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(54) **Title:** COMPOUNDS AND METHODS FOR TREATMENT AND DIAGNOSIS OF CHLAMYDIAL INFECTION

(57) **Abstract:** Compounds and methods for the diagnosis and treatment of Chlamydial infection are disclosed. The compounds provided include polypeptides that contain at least one antigenic portion of a *Chlamydia* antigen and DNA sequences encoding such polypeptides. Pharmaceutical compositions and vaccines comprising such polypeptides or DNA sequences are also provided, together with antibodies directed against such polypeptides. Diagnostic kits containing such polypeptides or DNA sequences and a suitable detection reagent may be used for the detection of Chlamydial infection in patients and in biological samples.

COMPOUNDS AND METHODS FOR TREATMENT AND DIAGNOSIS OF CHLAMYDIAL INFECTION

TECHNICAL FIELD

The present invention relates generally to the detection and treatment of Chlamydial infection. In particular, the invention is related to polypeptides comprising a *Chlamydia* antigen and the use of such polypeptides for the serodiagnosis and treatment of Chlamydial infection.

BACKGROUND OF THE INVENTION

Chlamydiae are intracellular bacterial pathogens that are responsible for a wide variety of important human and animal infections. *Chlamydia trachomatis* is one of the most common causes of sexually transmitted diseases and can lead to pelvic inflammatory disease (PID), resulting in tubal obstruction and infertility. *Chlamydia trachomatis* may also play a role in male infertility. In 1990, the cost of treating PID in the US was estimated to be $4 billion. Trachoma, due to ocular infection with *Chlamydia trachomatis*, is the leading cause of preventable blindness worldwide. *Chlamydia pneumonia* is a major cause of acute respiratory tract infections in humans and is also believed to play a role in the pathogenesis of atherosclerosis and, in particular, coronary heart disease. Individuals with a high titer of antibodies to *Chlamydia pneumonia* have been shown to be at least twice as likely to suffer from coronary heart disease as seronegative individuals. Chlamydial infections thus constitute a significant health problem both in the US and worldwide.

Chlamydial infection is often asymptomatic. For example, by the time a woman seeks medical attention for PID, irreversible damage may have already occurred resulting in infertility. There thus remains a need in the art for improved vaccines and pharmaceutical compositions for the prevention and treatment of *Chlamydia* infections. The present invention fulfills this need and further provides other related advantages.

SUMMARY OF THE INVENTION

The present invention provides compositions and methods for the diagnosis and therapy of *Chlamydia* infection. In one aspect, the present invention provides polypeptides comprising an immunogenic portion of a *Chlamydia* antigen, or a

variant of such an antigen. Certain portions and other variants are immunogenic, such that the ability of the variant to react with antigen-specific antisera is not substantially diminished. Within certain embodiments, the polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence seleeted from the group consisting of (a) a sequence of SEQ ID NO: 1-48, 114-121, and 125-138; (b) the complements of said sequences; and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions. In specific embodiments, the polypeptides of the present invention comprise at least a portion of a *Chlamydial* protein that includes an amino acid sequence selected from the group consisting of sequences recited in SEQ ID NO:122-124 and 139-140 and variants thereof.

The present invention further provides polynucleotides that eneode a polypeptide as described above, or a portion thereof (such as a portion encoding at least 15 amino acid residues of a *Chlamydial* protein), expression vectors comprising such polynucleotides and host cells transformed or transfeeted with such expression vectors.

In a related aspect, polynucleotide sequences encoding the above polypeptides, recombinant expression vectors comprising one or more of these polynucleotide sequences and host cells transformed or transfected with such expression vectors are also provided.

In another aspect, the present invention provides fusion proteins comprising an inventive polypeptide, or, alternatively, an inventive polypeptide and a known *Chlamydia* antigen, as well as polynucleotides encoding sueh fusion proteins, in combination with a physiologically acceptable carrier or immunostimulant for use as pharmaceutical compositions and vaccines thereof.

The present invention further provides pharmaceutical compositions that comprise: (a) an antibody, both polyclonal and monoclonal, or antigen-binding fragment thereof that specifically binds to a *Chlamydial* protein; and (b) a physiologically acceptable carrier. Within other aspects, the present invention provides pharmaeeutical compositions that comprise one or more *Chlamydia* polypeptides diselosed herein, for example, a polypeptide of SEQ ID NO: 95-109, 122-124 and 139-140, or a polynucleotide molecule encoding such a polypeptide, such as a polynucleotide sequence of SEQ ID NO: 1-48, 80-94, 114-121 and 125-138, and a physiologically acceptable carrier. The invention also provides compositions for

prophylactic and therapeutic purposes comprising one or more of the disclosed polynucleotides and/or polypeptides and an immunostimulant, e.g., an adjuvant.

In yet another aspect, methods are provided for stimulating an immune response in a patient, e.g., for inducing protective immunity in a patient, comprising administering to a patient an effective amount of one or more of the above pharmaceutical compositions or vaccines.

In yet a further aspect, methods for the treatment of *Chlamydia* infection in a patient are provided, the methods comprising obtaining peripheral blood mononuclear cells (PBMC) from the patient, incubating the PBMC with a polypeptide of the present invention (or a polynucleotide that encodes such a polypeptide) to provide incubated T cells and administering the incubated T cells to the patient. The present invention additionally provides methods for the treatment of *Chlamydia* infection that comprise incubating antigen presenting cells with a polypeptide of the present invention (or a polynucleotide that encodes such a polypeptide) to provide incubated antigen presenting cells and administering the incubated antigen presenting cells to the patient. Proliferated cells may, but need not, be cloned prior to administration to the patient. In certain embodiments, the antigen presenting cells are selected from the group consisting of dendritic cells, macrophages, monocytes, B-cells, and fibroblasts. Compositions for the treatment of *Chlamydia* infection comprising T cells or antigen presenting cells that have been incubated with a polypeptide or polynucleotide of the present invention are also provided. Within related aspects, vaccines are provided that comprise: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) an immunostimulant.

The present invention further provides, within other aspects, methods for removing *Chlamydial*-infected cells from a biological sample, comprising contacting a biological sample with T cells that specifically react with a *Chlamydial* protein, wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the protein from the sample.

Within related aspects, methods are provided for inhibiting the development of *Chlamydial* infection in a patient, comprising administering to a patient a biological sample treated as described above. In further aspects of the subject invention, methods and diagnostic kits are provided for detecting *Chlamydia* infection

in a patient. In one embodiment, the method comprises: (a) contacting a biological sample with at least one of the polypeptides or fusion proteins disclosed herein; and (b) detecting in the sample the presence of binding agents that bind to the polypeptide or fusion protein, thereby detecting *Chlamydia* infection in the biological sample. Suitable biological samples include whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid and urine. In one embodiment, the diagnostic kits comprise one or more of the polypeptides or fusion proteins disclosed herein in combination with a detection reagent. In yet another embodiment, the diagnostic kits comprise either a monoclonal antibody or a polyclonal antibody that binds with a polypeptide of the present invention.

The present invention also provides methods for detecting *Chlamydia* infection comprising: (a) obtaining a biological sample from a patient; (b) contacting the sample with at least two oligonucleotide primers in a polymerase chain reaction, at least one of the oligonucleotide primers being specific for a polynucleotide sequence disclosed herein; and (c) detecting in the sample a polynucleotide sequence that amplifies in the presence of the oligonucleotide primers. In one embodiment, the oligonucleotide primer comprises at least about 10 contiguous nucleotides of a polynucleotide sequence peptide disclosed herein, or of a sequence that hybridizes thereto.

In a further aspect, the present invention provides a method for detecting *Chlamydia* infection in a patient comprising: (a) obtaining a biological sample from the patient; (b) contacting the sample with an oligonucleotide probe specific for a polynucleotide sequence disclosed herein; and (c) detecting in the sample a polynucleotide sequence that hybridizes to the oligonucleotide probe. In one embodiment, the oligonucleotide probe comprises at least about 15 contiguous nucleotides of a polynucleotide sequence disclosed herein, or a sequence that hybridizes thereto.

These and other aspects of the present invention will become apparent upon reference to the following detailed description. All referenees disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

SEQUENCE IDENTIFIERS

SEQ ID NO:1 sets forth a DNA sequence identified for clone E4-A2-39 (CT10 positive) that is 1311 bp and contains the entire ORF for CT460 (SWIB) and a partial ORF for CT461 (yaeI).

SEQ ID NO:2 sets forth a DNA sequence for clone E2-B10-52 (CT10 positive) that has a 1516 bp insert that contains partial ORFs for genes CT827 (nrdA-ribonucleoside reductase large chain) and CT828 (ndrB-ribonucleoside reductase small chain). These genes as were not identified in a Ct L2 library screening.

SEQ ID NO:3 sets forth a DNA sequence for clone E1-B1-80 (CT10 positive) (2397bp) that contains partial ORFs for several genes, CT812 (pmpD), CT015 (phoH ATPase), CT016 (hypothetical protein) and pGp1-D (C. trachomatis plasmid gene).

SEQ ID NO:4 sets forth a DNA sequence for clone E4-F9-4 (CT10, CL8, CT1, CT5, CT13, and CHH037 positive) that contains a 1094 bp insert that has a partial ORF for the gene CT316 (L7/L12 ribosomal protein) as well as a partial ORF for gene CT315 (RNA polymerase beta).

SEQ ID NO:5 sets forth a DNA sequence for clone E2-H6-40 (CT3 positive) that has a 2129 bp insert that contains the entire ORF for the gene CT288 and very small fragments of genes CT287 and CT289. Genes in this clone have not been identified in screening with a Ct L2 library.

SEQ ID NO:6 sets forth a DNA sequence for clone E5-D4-2 (CT3, CT10, CT1, CT5, CT12, and CHH037 positive) that has a 1828 bp insert that contains a partial ORF for gene CT378 (pgi), complete ORF for gene CT377 (ltuA) and a complete ORF for the gene CT376 (malate dehydrogenase). In addition, the patient lines CT10, CT1, CT5, CT12, and CHH037 also identified this clone.

SEQ ID NO:7 sets forth a DNA sequence for clone E6-C1-31 (CT3 positive) that has a 861 bp insert that contains a partial ORF for gene CT858.

SEQ ID NO:8 sets forth a DNA sequence for clone E9-E11-76 (CT3 positive) that contains a 763 bp insert that is an amino terminal region of the gene for CT798 (Glycogen synthase). This gene was not identified in a previous screening with a Ct L2 library.

SEQ ID NO:9 sets forth a DNA sequence for clone E2-A9-26 (CT1-positive) that contains part of the gene for ORF-3 which is found on the plasmid in Chlamydia trachomatis.

SEQ ID NO:10 sets forth a DNA sequence for clone E2-G8-94 (CT1-positive) that has the carboxy terminal end of Lpda gene as well as a partial ORF for CT556.

SEQ ID NO: 11 sets forth a DNA sequence for clone E1-H1-14 (CT1 positive) that has a 1474 bp insert that contains the amino terminal part of an Lpda ORF on the complementary strand.

SEQ ID NO: 12 sets forth a DNA sequence for clone E1-A5-53 (CT1 positive) that contains a 2017 bp insert that has an amino terminal portion of the ORF for dnaK gene on the complementary strand, a partial ORF for the grpE gene (CT395) and a partial ORF for CT166 .

SEQ ID NO: 13 sets forth a DNA sequence for clone E3-A1-50 (positive on CT1 line) that is 1199 bp and contains a carboxy terminal portion of the ORF for CT622.

SEQ ID NO: 14 sets forth a DNA sequence for clone E3-E2-22 that has 877 bp, containing a complete ORF for CT610 on the complementary strand, and was positive on both CT3 and CT10 lines.

SEQ ID NO: 15 sets forth the DNA sequence for clone E5-E2-10 (CT10 positive) which is 427 bp and contains a partial ORF for the major outer membrane protein omp1.SEQ ID NO: 16 sets forth the DNA sequence for clone E2-D5-89 (516bp) which is a CT10 positive clone that contains a partial ORF for pmpD gene (CT812).

SEQ ID NO: 17 sets forth the DNA sequence for clone E4-G9-75 (CT10 positive) which is 723 bp and contains a partial ORF for the amino terminal region of the pmpH gene (CT872).

SEQ ID NO: 18 sets forth the DNA sequence for clone E3-F2-37 (CT10, CT3, CT11, and CT13 positive-1377bp insert) which contains a partial ORF for the tRNA-Trp (CT322) gene and a complete ORF for the gene secE (CT321).

SEQ ID NO: 19 sets forth the DNA sequence for clone E5-A11-8 (CT10 positive-1736 bp) which contains the complete ORF for groES (CT111) and a majority of the ORF for groEL (CT110).

SEQ ID NO: 20 sets forth the DNA sequence for clone E7-H11-61 (CT3 positive-1135 bp) which has partial inserts for fliA (CT061), tyrS (CT062), TSA (CT603) and a hypothetical protein (CT602).

SEQ ID NO: 21 sets forth a DNA sequence for clone E6-C8-95 which contains a 731 bp insert that was identified using the donor lines CT3, CT1, and CT12 line. This insert has a carboxy terminal half for the gene for the 60 kDa ORF.

SEQ ID NO: 22 sets forth the DNA sequence for clone E4-D2-79 (CT3 positive) which contains a 1181 bp insert that is a partial ORF for nrdA gene. The ORF for this gene was also identified from clone E2-B10-52 (CT10 positive).

SEQ ID NO: 23 sets forth the DNA sequence for clone E1-F9-79 (167 bp; CT11 positive) which contains a partial ORF for the gene CT133 on the complementary strand. CT133 is a predicted rRNA methylase.

SEQ ID NO: 24 sets forth the DNA sequence for clone E2-G12-52 (1265 bp; CT11 positive) which contains a partial ORF for clpB, a protease ATPase.

SEQ ID NO: 25 sets forth the DNA sequence for clone E4-H3-56 (463 bp insert; CT1 positive) which contains a partial ORF for the TSA gene (CT603) on the complementary strand.

SEQ ID NO: 26 sets forth the DNA sequence for clone E5-E9-3 (CT1 positive) that contains a 636 bp insert partially encoding the ORF for dnaK like gene. Part of this sequence was also identified in clone E1-A5-53.

SEQ ID NO:27 sets forth the full-length serovar E DNA sequence of CT875.

SEQ ID NO:28 sets for the full-length serovar E DNA sequence of CT622.

SEQ ID NO:29 sets forth the DNA sequence for clone E3-B4-18 (CT1 positive) that contains a 1224 bp insert containing 4 ORFs. The complete ORF for CT772, and the partial ORFs of CT771, CT191, and CT190.

SEQ ID NO:30 sets forth the DNA sequence for the clone E9-E10-51 (CT10 positive) that contains an 883 bp insert containing two partial ORF, CT680 and CT679.

SEQ ID NO:31 sets forth the DNA sequence of the clone E9-D5-8 (CT10, CTCT1, CT4, and CT11 positive) that contains a393 bp insert containing the partial ORF for CT680.

SEQ ID NO:32 sets forth the DNA sequence of the clone E7-B1-16 (CT10, CT3, CT5, CT11, CT13, and CHH037 positive) that contains a 2577 bp insert containing three ORFs, two full length ORFs for CT694 and CT695 and the third containing the N-terminal portion of CT969.

SEQ ID NO:33 sets forth the DNA sequence of the clone E9-G2-93 (CT10 positive) that contains a 554 bp insert containing a partial ORF for CT178.

SEQ ID NO:34 sets forth the DNA sequence of the clone E5-A8-85 (CT1 positive) that contains a 1433 bp insert containing two partial ORFs for CT875 and CT001.

SEQ ID NO:35 sets forth the DNA sequence of the clone E10-C6-45 (CT3 positive) that contains a 196 bp insert containing a partial ORF for CT827.

SEQ ID NO:36 sets forth the DNA sequence of the clone E7-H11-10 (CT3 positive) that contains a 1990 bp insert containing the partial ORFs of CT610 and CT613 and the complete ORFs of CT611 and CT612.

SEQ ID NO:37 sets forth the DNA sequence of the clone E2-F7-11 (CT3 and CT10 positive) that contains a 2093 bp insert. It contains a large region of CT609, a complete ORF for CT610 and a partial ORF for CT611.

SEQ ID NO:38 sets forth the DNA sequence of the cloneE3-A3-31 (CT1 positive) that contains an 1834 bp insert containing a large region of CT622.

SEQ ID NO:39 sets forth the DNA sequence of the clone E1-G9-23 (CT3 positive) that contains an 1180 bp insert containing almost the entire ORF for CT798.

SEQ ID NO:40 sets forth the DNA sequence of the clone E4-D6-21 (CT 3 positive) that contains a 1297 bp insert containing the partial ORFs of CT329 and CT327 and the complete ORF of CT328.

SEQ ID NO:41 sets forth the DNA sequence of the clone E3-F3-18 (CT1 positive) that contains an 1141 bp insert containing the partial ORF of CT871.

SEQ ID NO:42 sets forth the DNA sequence of the clone E10-B2-57 (CT10 positive) that contains an 822 bp insert containing the complete ORF of CT066.

SEQ ID NO:43 sets forth the DNA sequence of the clone E3-F3-7 (CT1 positive) that contains a 1643 bp insert containing the partial ORFs of CT869 and CT870.

SEQ ID NO:44 sets forth the DNA sequence of the clone E10-H8-1 (CT3 and CT10 positive) that contains an 1862 bp insert containing the partial ORFs of CT871 and CT872.

SEQ ID NO:45 sets forth the DNA sequence of the clone E3-D10-46 (CT1, CT3, CT4, CT11, and CT12 positive) that contains a 1666 bp insert containing the partial ORFs for CT770 and CT773 and the complete ORFs for CT771 and CT722.

SEQ ID NO:46 sets forth the DNA sequence of the clone E2-D8-19 (CT1 positive) that contains a 2010 bp insert containing partial ORFs, ORF3 and ORF6, and complete ORFs, ORF4 and ORF5.

SEQ ID NO:47 sets forth the DNA sequence of the clone E4-C3-40 (CT10 positive) that contains a 2044 bp insert containing the partial ORF for CT827 and a complete ORF for CT828.

SEQ ID NO:48 sets forth the DNA sequence of the clone E3-H6-10 (CT12 positive) that contains a 3743 bp insert containing the partial ORFs for CT223 and CT229 and the complete ORFs for CT224 and CT224, CT225, CT226, CT227, and CT228.

SEQ ID NO:49 sets forth the DNA sequence for the Chlamydia pneumoniae homologue, CPn0454 of the Chlamydia trachomatis gene CT872.

SEQ ID NO:50 sets forth the DNA sequence for the Chlamydia pneumoniae homologue, CPn0187, of the Chlamydia trachomatis gene CT133.

SEQ ID NO:51 sets forth the DNA sequence for the Chlamydia pneumoniae homologue, CPn0075 of the Chlamydia trachomatis gene CT321.

SEQ ID NO:52 sets forth the DNA sequence for the Chlamydia pneumoniae homologue, CPn0074, of the Chlamydia trachomatis gene CT322.

SEQ ID NO:53 sets forth the DNA sequenee for the Chlamydia pneumoniae homologue, CPn0948, of the Chlamydia trachomatis gene CT798.

SEQ ID NO:54 sets forth the DNA sequence for the Chlamydia pneumoniae homologue, CPn0985, of the Chlamydia trachomatis gene CT828.

SEQ ID NO:55 sets forth the DNA sequence for the Chlamydia pneumoniae homologue, CPn0984, of the Chlamydia trachomatis gene CT827.

SEQ ID NO:56 sets forth the DNA sequence for the Chlamydia pneumoniae homologue, CPn0062, of the Chlamydia trachomatis gene CT289.

SEQ ID NO:57 sets forth the DNA sequence for the Chlamydia pneumoniae homologue, CPn00065, of the Chlamydia trachomatis gene CT288.

SEQ ID NO:58 sets forth the DNA sequence for the Chlamydia pneumoniae homologue, CPn0438, of the Chlamydia trachomatis gene CT287.

SEQ ID NO:59 sets forth the DNA sequence for the Chlamydia pneumoniae homologue, CPn0963, of the Chlamydia trachomatis gene CT812.

SEQ ID NO:60 sets forth the DNA sequence for the Chlamydia pneumoniae homologue, CPn0778, of the Chlamydia trachomatis gene CT603.

SEQ ID NO:61 sets forth the DNA sequence for the Chlamydia pneumoniae homologue, CPn0503, of the Chlamydia trachomatis gene CT396.

SEQ ID NO:62 sets forth the DNA sequence for the Chlamydia pneumoniae homologue, CPn1016, of the Chlamydia trachomatis gene CT858.

SEQ ID NO:63 sets forth the DNA sequence for the Chlamydia pneumoniae homologue, CPn0728, of the Chlamydia trachomatis gene CT622.

SEQ ID NO:64 sets forth the DNA sequence for the Chlamydia pneumoniae homologue, CPn0557, of the Chlamydia trachomatis gene CT460.

SEQ ID NO:65 sets forth the amino acid sequence for the Chlamydia pneumoniae homologue, CPn0454, of the Chlamydia trachomatis gene CT872.

SEQ ID NO:66 sets forth the amino acid sequence for the Chlamydia pneumoniae homologue, CPn0187, of the Chlamydia trachomatis gene CT133.

SEQ ID NO:67 sets forth the amino acid sequence for the Chlamydia pneumoniae homologue, CPn0075, of the Chlamydia trachomatis gene CT321.

SEQ ID NO:68 sets forth the amino acid sequence for the Chlamydia pneumoniae homologue, CPn0074, of the Chlamydia trachomatis gene CT322.

SEQ ID NO:69 sets forth the amino acid sequence for the Chlamydia pneumoniae homologue, CPn0948, of the Chlamydia trachomatis gene CT798.

SEQ ID NO:70 sets forth the amino acid sequence for the Chlamydia pneumoniae homologue, CPn0985, of the Chlamydia trachomatis gene CT828.

SEQ ID NO:71 sets forth the amino acid sequence for the Chlamydia pneumoniae homologue, CPn0984, of the Chlamydia trachomatis gene CT827.

SEQ ID NO:72 sets forth the amino acid sequence for the Chlamydia pneumoniae homologue, CPn0062, of the Chlamydia trachomatis gene CT289.

SEQ ID NO:73 sets forth the amino acid sequence for the Chlamydia pneumoniae homologue, CPn0065, of the Chlamydia trachomatis gene CT288.

SEQ ID NO:74 sets forth the amino aeid sequence for the Chlamydia pneumoniae homologue, CPn0438, of the Chlamydia trachomatis gene CT287.

SEQ ID NO:75 sets forth the amino acid sequence for the Chlamydia pneumoniae homologue, CPn0963, of the Chlamydia trachomatis gene CT812.

SEQ ID NO:76 sets forth the amino acid sequence for the Chlamydia pneumoniae homologue, CPn0778, of the Chlamydia trachomatis gene CT603.

SEQ ID NO:77 sets forth the amino acid sequence for the Chlamydia pneumoniae homologue, CPn1016, of the Chlamydia trachomatis gene CT858.

SEQ ID NO:78 sets forth the amino acid sequence for the Chlamydia pneumoniae homologue, CPn0728, of the Chlamydia trachomatis gene CT622.

SEQ ID NO:79 sets forth the amino acid sequence for the Chlamydia pneumoniae homologue, CPn0557, of the Chlamydia trachomatis gene CT460.

SEQ ID NO:80 sets forth the full-length serovar D DNA sequenee of the Chlamydia trachomatis gene CT872.

SEQ ID NO:81 sets forth the full-length serovar D DNA sequence of the Chlamydia trachomatis gene CT828.

SEQ ID NO:82 sets forth the full-length serovar D DNA sequence of the Chlamydia trachomatis gene CT827.

SEQ ID NO:83 sets forth the full-length serovar D DNA sequence of the Chlamydia traehomatis gene CT812.

SEQ ID NO:84 sets forth the full-length serovar D DNA sequence of the Chlamydia trachomatis gene CT798.

SEQ ID NO:85 sets forth the full-length serovar D DNA sequence of the Chlamydia trachomatis gene CT681 (MompF).

SEQ ID NO:86 sets forth the full-length serovar D DNA sequence of the Chlamydia trachomatis gene CT603.

SEQ ID NO:87 sets forth the full-length serovar D DNA sequenee of the Chlamydia trachomatis gene CT460.

SEQ ID NO:88 sets forth the full-length serovar D DNA sequence of the Chlamydia trachomatis gene CT322.

SEQ ID NO:89 sets forth the full-length serovar D DNA sequence of the Chlamydia trachomatis gene CT321.

SEQ ID NO:90 sets forth the full-length serovar D DNA sequence of the Chlamydia trachomatis gene CT289.

SEQ ID NO:91 sets forth the full-length serovar D DNA sequence of the Chlamydia traehomatis gene CT288.

SEQ ID NO:92 sets forth the full-length serovar D DNA sequence of the Chlamydia trachomatis gene CT287.

SEQ ID NO:93 sets forth the full-length serovar D DNA sequence of the Chlamydia trachomatis gene CT133.

SEQ ID NO:94 sets forth the full-length serovar D DNA sequence of the Chlamydia trachomatis gene CT113.

SEQ ID NO:95 sets forth the full-length serovar D amino acid sequence of the Chlamydia trachomatis gene CT872.

SEQ ID NO:96 sets forth the full-length serovar D amino acid sequence of the Chlamydia trachomatis gene CT828.

SEQ ID NO:97 sets forth the full-length serovar D amino acid sequence of the Chlamydia trachomatis gene CT827.

SEQ ID NO:98 sets forth the full-length serovar D amino acid sequence of the Chlamydia trachomatis gene CT812.

SEQ ID NO:99 sets forth the full-length serovar D amino acid sequence of the Chlamydia trachomatis gene CT798.

SEQ ID NO:100 sets forth the full-length serovar D amino acid sequence of the Chlamydia trachomatis gene CT681.

SEQ ID NO:101 sets forth the full-length serovar D amino acid sequenee of the Chlamydia trachomatis gene CT603.

SEQ ID NO:102 sets forth the full-length serovar D amino acid sequence of the Chlamydia trachomatis gene CT460.

SEQ ID NO:103 sets forth the full-length serovar D amino acid sequence of the Chlamydia trachomatis gene CT322.

SEQ ID NO:104 sets forth the full-length serovar D amino acid sequence of the Chlamydia trachomatis gene CT321.

SEQ ID NO:105 sets forth the full-length serovar D amino acid sequence of the Chlamydia trachomatis gene CT289.

SEQ ID NO:106 sets forth the full-length serovar D amino acid sequence of the Chlamydia trachomatis gene CT288.

SEQ ID NO:107 sets forth the full-length serovar D amino acid sequence of the Chlamydia trachomatis gene CT287.

SEQ ID NO:108 sets forth the full-length serovar D amino acid sequence of the Chlamydia trachomatis gene CT133.

SEQ ID NO:109 sets forth the full-length serovar D amino acid sequence of the Chlamydia trachomatis gene CT113.

SEQ ID NO:110 sets forth the DNA sequence for the Chlamydia pneumoniae homologue, CPn0695, of the Chlamydia trachomatis gene CT681.

SEQ ID NO:111 sets forth the DNA sequence for the Chlamydia pneumoniae homologue, CPn0144, of the Chlamydia trachomatis gene CT113.

SEQ ID NO:112 sets forth the amino acid sequence for the Chlamydia pneumoniae homologue, CPn0695, of the Chlamydia trachomatis gene CT681.

SEQ ID NO:113 sets forth the amino acid sequence for the Chlamydia pneumoniae homologue, CPn0144, of the Chlamydia trachomatis gene CT113.

SEQ ID NO:114 sets forth the DNA sequence of the clone E7-B12-65 (CHH037 positive) that contains a 1179 bp insert containing complete ORF for 376.

SEQ ID NO:115 sets forth the DNA sequenee of the clone E4-H9-83 (CHH037 positive) that contains the partial ORF for the heat shock protein GroEL (CT110).

SEQ ID NO:116 sets forth the DNA sequence of the clone E9-B10-52 (CHH037 positive) that contains the partial ORF for the the gene yscC (CT674).

SEQ ID NO:117 sets forth the DNA sequence of the clone E7-A7-79 (CHH037 positive) that contains the complete ORF for the histone like development

gene hctA (CT743) and a partial ORF for the rRNA methyltransferase gene ygcA (CT742).

SEQ ID NO:118 sets forth the DNA sequence of the clone E2-D11-18 (CHH037 positive) that contains the partial ORF for hctA (CT743).

SEQ ID NO:119 sets forth the DNA sequence for the Chlamydia traehomatis serovar E hypothetical protein CT694.

SEQ ID NO:120 sets forth the DNA sequence for the Chlamydia trachomatis serovar E hypothetical protein CT695.

SEQ ID NO:121 sets forth the DNA sequence for the Chlamydia trachomatis serovar E L1 ribosomal protein.

SEQ ID NO:122 sets forth the amino acid sequence for the Chlamydia trachomatis serovar E hypothetical protein CT694.

SEQ ID NO:123 sets forth the amino acid sequenee for the Chlamydia trachomatis serovar E hypothetical protein CT695.

SEQ ID NO:124 sets forth the amino acid sequence for the Chlamydia trachomatis serovar E L1 ribosomal protein.

SEQ ID NO:125 sets forth the DNA sequence of the clone E9-H6-15 (CT3 positive) that contains the partial ORF for the pmpB gene (CT413).

SEQ ID NO:126 sets forth the DNA sequence of the clone E3-D10-87 (CT1 positive) that contains the partial ORFs for the hypothetical genes CT388 and CT389.

SEQ ID NO:127 sets forth the DNA sequence of the clone E9-D6-43 (CT3 positive) that contains the partial ORF for the CT858.

SEQ ID NO:128 sets forth the DNA sequence of the clone E3-D10-4 (CT1 positive) that contains the partial ORF for pGP3-D, an ORF encoded on the plasmid pCHL1.

SEQ ID NO:129 sets forth the DNA sequence of the clone E3-G8-7 (CT1 positive) that contains the partial ORFs for the CT557 (LpdA) and CT558 (LipA).

SEQ ID NO:130 sets forth the DNA sequence of the clone E3-F11-32 (CT1 positive) that contains the partial ORF for pmpD (CT812).

SEQ ID NO:131 sets forth the DNA sequence of the clone E2-F8-5 (CT12 positive) that contains the complete ORF for the 15 kDa ORF (CT442) and a partial ORF for the 60kDa ORF (CT443).

SEQ ID NO:132 sets forth the DNA sequence of the clone E2-G4-39 (CT12 positive) that contains the partial ORF for the 60kDa ORF (CT443).

SEQ ID NO:133 sets forth the DNA sequence of the clone E9-D1-16 (CT10 positive) that contains the partial ORF for pmpH (CT872).

SEQ ID NO:134 sets forth the DNA sequence of the clone E3-F3-6 (CT1 positive) that contains the partial ORFs for the genes accB (CT123), L1 ribosomal (CT125) and S9 ribosomal (CT126).

SEQ ID NO:135 sets forth the DNA sequence of the clone E2-D4-70 (CT12 positive) that contains the partial ORF for the pmpC gene (CT414).

SEQ ID NO:136 sets forth the DNA sequence of the clone E5-A1-79 (CT1 positive) that contains the partial ORF for ydhO (CT127), a complete ORF for S9 ribosomal gene (CT126), a complete ORF for the L1 ribosomal gene (CT125) and a partial ORF for accC (CT124).

SEQ ID NO:137 sets forth the DNA sequence of the clone E1-F7-16 (CT12, CT3, and CT11 positive) that contains the partial ORF for the ftsH gene (CT841) and the entire ORF for the pnp gene (CT842).

SEQ ID NO:138 sets forth the DNA sequence of the clone E1-D8-62 (CT12 positive) that contains the partial ORFs for the ftsH gene (CT841) and for the pnp gene (CT842).

SEQ ID NO:139 sets forth the amino acid sequence for the serovar E protein CT875.

SEQ ID NO:140 sets forth the amino acid sequence for the serovar E protein CT622.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

As noted above, the present invention is generally directed to compositions and methods for the diagnosis and treatment of Chlamydial infection. In one aspect, the compositions of the subject invention include polypeptides that comprise at least one immunogenic portion of a *Chlamydia* antigen, or a variant thereof.

In specific embodiments, the subject invention discloses polypeptides comprising an immunogenic portion of a *Chlamydia* antigen, wherein the *Chlamydia* antigen comprises an amino acid sequence encoded by a polynucleotide molecule including a sequence selected from the group consisting of (a) nucleotide sequences recited in SEQ ID NO:1-48, 114-121, and 125-138 (b) the complements of said nucleotide sequences, and (c) variants of such sequences.

POLYNUCLEOTIDE COMPOSITIONS

As used herein, the terms "DNA segment" and "polynucleotide" refer to a DNA molecule that has been isolated free of total genomic DNA of a particular species. Therefore, a DNA segment encoding a polypeptide refers to a DNA segment that contains one or more coding sequences yet is substantially isolated away from, or purified free from, total genomic DNA of the species from which the DNA segment is obtained. Included within the terms "DNA segment" and "polynucleotide" are DNA segments and smaller fragments of such segments, and also recombinant vectors, including, for example, plasmids, cosmids, phagemids, phage, viruses, and the like.

As will be understood by those skilled in the art, the DNA segments of this invention can include genomic sequences, extra-genomic and plasmid-encoded sequences and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides, peptides and the like. Such segments may be naturally isolated, or modified synthetically by the hand of man.

"Isolated," as used herein, means that a polynucleotide is substantially away from other coding sequences, and that the DNA segment does not contain large portions of unrelated coding DNA, such as large chromosomal fragments or other functional genes or polypeptide coding regions. Of course, this refers to the DNA segment as originally isolated, and does not exclude genes or coding regions later added to the segment by the hand of man.

As will be recognized by the skilled artisan, polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present

invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

Polynucleotides may comprise a native Chlamydia sequence or may comprise a variant, or a biological or antigenic functional equivalent of such a sequence. Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions, as further described below, preferably such that the immunogenicity of the encoded polypeptide is not diminished, relative to a native Chlamydia protein. The effect on the immunogenicity of the encoded polypeptide may generally be assessed as described herein. The term "variants" also encompasses homologous genes of xenogenic origin.

When comparing polynucleotide or polypeptide sequences, two sequences are said to be "identical" if the sequence of nucleotides or amino acids in the two sequences is the same when aligned for maximum correspondence, as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins – Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) Unified Approach to Alignment and Phylogenes pp. 626-645 *Methods in Enzymology* vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M. (1989) *CABIOS 5*:151-153; Myers, E.W. and Muller W. (1988) *CABIOS 4*:11-17; Robinson, E.D. (1971) *Comb. Theor 11*:105; Santou, N. Nes, M. (1987) *Mol. Biol. Evol. 4*:406-425; Sneath, P.H.A. and Sokal, R.R. (1973) *Numerical Taxonomy – the Principles and Practice of Numerical Taxonomy*, Freeman Press, San Francisco, CA; Wilbur, W.J. and

Lipman, D.J. (1983) *Proc. Natl. Acad., Sci. USA 80*:726-730.

Alternatively, optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman (1981) *Add. APL. Math* 2:482, by the identity alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443, by the search for similarity methods of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. USA* 85: 2444, by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection.

One preferred example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, whieh are described in Altschul et al. (1977) *Nucl. Acids Res.* 25:3389-3402 and Altschul et al. (1990) *J. Mol. Biol.* 215:403-410, respectively. BLAST and BLAST 2.0 can be used, for example with the parameters described herein, to determine percent sequence identity for the polynucleotides and polypeptides of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. In one illustrative example, cumulative scores can be calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix can be used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-seoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915) alignments, (B) of 50, expectation (E) of 10, M=5, N=-4 and a comparison of both strands.

Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide or polypeptide sequence in the comparison

window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (*i.e.*, the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

Therefore, the present invention encompasses polynucleotide and polypeptide sequences having substantial identity to the sequences diselosed herein, for example those comprising at least 50% sequence identity, preferably at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% or higher, sequence identity compared to a polynucleotide or polypeptide sequence of this invention using the methods described herein, (e.g., BLAST analysis using standard parameters, as described below). One skilled in this art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like.

In additional embodiments, the present invention provides isolated polynucleotides and polypeptides comprising various lengths of contiguous stretches of sequence identical to or complementary to one or more of the sequences disclosed herein. For example, polynucleotides are provided by this invention that comprise at least about 15, 20, 30, 40, 50, 75, 100, 150, 200, 300, 400, 500 or 1000 or more contiguous nucleotides of one or more of the sequences disclosed herein as well as all intermediate lengths there between. It will be readily understood that "intermediate lengths", in this eontext, means any length between the quoted values, such as 16, 17, 18, 19, *etc.*; 21, 22, 23, *etc.*; 30, 31, 32, *etc.*; 50, 51, 52, 53, *etc.*; 100, 101, 102, 103, *etc.*; 150, 151, 152, 153, *etc.*; including all integers through 200-500; 500-1,000, and the like.

The polynucleotides of the present invention, or fragments thereof, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme

sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protoeol. For example, illustrative DNA segments with total lengths of about 10,000, about 5000, about 3000, about 2,000, about 1,000, about 500, about 200, about 100, about 50 base pairs in length, and the like, (including all intermediate lengths) are contemplated to be useful in many implementations of this invention.

In other embodiments, the present invention is directed to polynucleotides that are capable of hybridizing under moderately stringent conditions to a polynucleotide sequence provided herein, or a fragment thereof, or a complementary sequence thereof. Hybridization techniques are well known in the art of molecular biology. For purposes of illustration, suitable moderately stringent conditions for testing the hybridization of a polynucleotide of this invention with other polynucleotides include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS.

Moreover, it will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetie code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynueleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention. Further, alleles of the genes comprising the polynueleotide sequences provided herein are within the scope of the present invention. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or function. Alleles may be identified using standard techniques (such as hybridization, amplification and/or database sequence comparison).

PROBES AND PRIMERS

In other embodiments of the present invention, the polynucleotide sequences provided herein can be advantageously used as probes or primers for nucleic acid hybridization. As such, it is contemplated that nucleic acid segments that comprise a sequence region of at least about 15 nucleotide long contiguous sequence that has the same sequence as, or is complementary to, a 15 nucleotide long contiguous sequence disclosed herein will find particular utility. Longer contiguous identical or complementary sequences, *e.g.*, those of about 20, 30, 40, 50, 100, 200, 500, 1000 (including all intermediate lengths) and even up to full length sequences will also be of use in certain embodiments.

The ability of such nucleic acid probes to specifically hybridize to a sequence of interest will enable them to be of use in detecting the presence of complementary sequences in a given sample. However, other uses are also envisioned, such as the use of the sequence information for the preparation of mutant species primers, or primers for use in preparing other genetic constructions.

Polynucleotide molecules having sequence regions consisting of contiguous nucleotide stretches of 10-14, 15-20, 30, 50, or even of 100-200 nucleotides or so (including intermediate lengths as well), identical or complementary to a polynucleotide sequence disclosed herein, are particularly contemplated as hybridization probes for use in, *e.g.*, Southern and Northern blotting. This would allow a gene product, or fragment thereof, to be analyzed, both in diverse cell types and also in various bacterial cells. The total size of fragment, as well as the size of the complementary stretch(es), will ultimately depend on the intended use or application of the particular nucleic acid segment. Smaller fragments will generally find use in hybridization embodiments, wherein the length of the contiguous complementary region may be varied, such as between about 15 and about 100 nucleotides, but larger contiguous complementarity stretches may be used, according to the length complementary sequences one wishes to detect.

The use of a hybridization probe of about 15-25 nucleotides in length allows the formation of a duplex molecule that is both stable and selective. Molecules having contiguous complementary sequences over stretches greater than 15 bases in length are generally preferred, though, in order to increase stability and selectivity of the

hybrid, and thereby improve the quality and degree of specific hybrid molecules obtained. One will generally prefer to design nucleic acid molecules having gene-complementary stretches of 15 to 25 contiguous nucleotides, or even longer where desired.

Hybridization probes may be selected from any portion of any of the sequences disclosed herein. All that is required is to review the sequence set forth in SEQ ID NO:1-48, 114-121, and 125-138, or to any continuous portion of the sequence, from about 15-25 nucleotides in length up to and including the full length sequence, that one wishes to utilize as a probe or primer. The choice of probe and primer sequences may be governed by various factors. For example, one may wish to employ primers from towards the termini of the total sequence.

Small polynucleotide segments or fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, as is commonly practiced using an automated oligonucleotide synthesizer. Also, fragments may be obtained by application of nucleic acid reproduction technology, such as the PCR™ technology of U. S. Patent 4,683,202 (incorporated herein by reference), by introducing selected sequences into recombinant vectors for recombinant production, and by other recombinant DNA techniques generally known to those of skill in the art of molecular biology.

The nucleotide sequences of the invention may be used for their ability to selectively form duplex molecules with complementary stretches of the entire gene or gene fragments of interest. Depending on the application envisioned, one will typically desire to employ varying conditions of hybridization to achieve varying degrees of selectivity of probe towards target sequence. For applications requiring high selectivity, one will typically desire to employ relatively stringent conditions to form the hybrids, *e.g.*, one will select relatively low salt and/or high temperature conditions, such as provided by a salt concentration of from about 0.02 M to about 0.15 M salt at temperatures of from about 50°C to about 70°C. Such selective conditions tolerate little, if any, mismatch between the probe and the template or target strand, and would be particularly suitable for isolating related sequences.

Of course, for some applications, for example, where one desires to prepare mutants employing a mutant primer strand hybridized to an underlying

template, less stringent (reduced stringency) hybridization conditions will typieally be needed in order to allow formation of the heteroduplex. In these circumstances, one may desire to employ salt conditions such as those of from about 0.15 M to about 0.9 M salt, at temperatures ranging from about 20°C to about 55°C. Cross-hybridizing species can thereby be readily identified as positively hybridizing signals with respect to control hybridizations. In any ease, it is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide, which serves to destabilize the hybrid duplex in the same manner as increased temperature. Thus, hybridization conditions can be readily manipulated, and thus will generally be a method of choice depending on the desired results.

POLYNUCLEOTIDE IDENTIFICATION AND CHARACTERIZATION

Polynucleotides may be identified, prepared and/or manipulated using any of a variety of well established techniques. For example, a polynucleotide may be identified, by screening a microarray of cDNAs for Chlamydia expression. Such screens may be performed, for example, using a Synteni microarray (Palo Alto, CA) according to the manufacturer's instructions (and essentially as described by Schena et al., *Proc. Natl. Acad. Sci. USA 93*:10614-10619, 1996 and Heller et al., *Proc. Natl. Acad. Sci. USA 94*:2150-2155, 1997). Alternatively, polynucleotides may be amplified from cDNA prepared from cells expressing the proteins described herein. Such polynucleotides may be amplified via polymerase chain reaction (PCR). For this approach, sequence-specific primers may be designed based on the sequenees provided herein, and may be purchased or synthesized.

An amplified portion of a polynucleotide of the present invention may be used to isolate a full length gene from a suitable library (*e.g.,* Chlamydia eDNA library) using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences.

For hybridization techniques, a partial sequence may be labeled (*e.g.,* by nick-translation or end-labeling with ^{32}P) using well known techniques. A bacterial or

bacteriophage library is then generally screened by hybridizing filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe (*see* Sambrook et al., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989). Hybridizing colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. cDNA clones may be analyzed to determine the amount of additional sequence by, for example, PCR using a primer from the partial sequence and a primer from the veetor. Restriction maps and partial sequences may be generated to identify one or more overlapping clones. The complete sequence may then be determined using standard techniques, which may involve generating a series of deletion clones. The resulting overlapping sequences can then assembled into a single contiguous sequence. A full length cDNA molecule ean be generated by ligating suitable fragments, using well known techniques.

Alternatively, there are numerous amplification techniques for obtaining a full length coding sequence from a partial cDNA sequence. Within such teehniques, amplifieation is generally performed via PCR. Any of a variety of commercially available kits may be used to perform the amplification step. Primers may be designed using, for example, software well known in the art. Primers are preferably 22-30 nucleotides in length, have a GC content of at least 50% and anneal to the target sequence at temperatures of about 68°C to 72°C. The amplified region may be sequenced as described above, and overlapping sequences assembled into a contiguous sequence.

One such amplification technique is inverse PCR (*see* Triglia et al., *Nucl. Acids Res. 16*:8186, 1988), which uses restrietion enzymes to generate a fragment in the known region of the gene. The fragment is then circularized by intramolecular ligation and used as a template for PCR with divergent primers derived from the known region. Within an alternative approach, sequences adjacent to a partial sequence may be retrieved by amplification with a primer to a linker sequence and a primer specific to a known region. The amplified sequences are typically subjected to a second round of amplifieation with the same linker primer and a second primer specific to the known region. A variation on this procedure, which employs two primers that initiate extension in opposite directions from the known sequence, is described in WO 96/38591. Another such technique is known as "rapid amplification of cDNA ends" or

RACE. This technique involves the use of an internal primer and an external primer, which hybridizes to a polyA region or vector sequence, to identify sequences that are 5' and 3' of a known sequence. Additional techniques include capture PCR (Lagerstrom et al., *PCR Methods Applic. 1*:111-19, 1991) and walking PCR (Parker et al., *Nucl. Acids. Res. 19*:3055-60, 1991). Other methods employing amplification may also be employed to obtain a full length eDNA sequence.

In certain instances, it is possible to obtain a full length cDNA sequenee by analysis of sequences provided in an expressed sequence tag (EST) database, such as that available from GenBank. Searches for overlapping ESTs may generally be performed using well known programs (*e.g.,* NCBI BLAST searches), and such ESTs may be used to generate a contiguous full length sequence. Full length DNA sequences may also be obtained by analysis of genomic fragments.

POLYNUCLEOTIDE EXPRESSION IN HOST CELLS

In other embodiments of the invention, polynucleotide sequences or fragments thereof which encode polypeptides of the invention, or fusion proteins or functional equivalents thereof, may be used in recombinant DNA molecules to direet expression of a polypeptide in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences that encode substantially the same or a functionally equivalent amino acid sequence may be produced and these sequences may be used to clone and express a given polypeptide.

As will be understood by those of skill in the art, it may be advantageous in some instances to produce polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally oceurring sequence.

Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene

fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences may be ligated to a heterologous sequence to encode a fusion protein. For example, to screen peptide libraries for inhibitors of polypeptide activity, it may be useful to encode a chimeric protein that can be recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the polypeptide-encoding sequence and the heterologous protein sequence, so that the polypeptide may be cleaved and purified away from the heterologous moiety.

Sequences encoding a desired polypeptide may be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers, M. H. et al. (1980) *Nucl. Acids Res. Symp. Ser.* 215-223, Horn, T. et al. (1980) *Nucl. Acids Res. Symp. Ser.* 225-232). Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of a polypeptide, or a portion thereof. For example, peptide synthesis can be performed using various solid-phase techniques (Roberge, J. Y. et al. (1995) *Science 269*:202-204) and automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin Elmer, Palo Alto, CA).

A newly synthesized peptide may be substantially purified by preparative high performance liquid chromatography (e.g., Creighton, T. (1983) Proteins, Structures and Molecular Principles, WH Freeman and Co., New York, N.Y.) or other comparable techniques available in the art. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure). Additionally, the amino acid sequence of a polypeptide, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

In order to express a desired polypeptide, the nucleotide sequences encoding the polypeptide, or functional equivalents, may be inserted into appropriate expression

vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art may be used to eonstruct expression vectors containing sequences encoding a polypeptide of interest and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA teehniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described in Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview, N.Y., and Ausubel, F. M. et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York. N.Y.

A variety of expression vector/host systems may be utilized to contain and express polynucleotide sequences. These include, but are not limited to, microorganisms sueh as bacteria transformed with recombinant bacteriophage, plasmid, or eosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems.

The "control elements" or "regulatory sequenees" present in an expression vector are those non-translated regions of the vector--enhancers, promoters, 5' and 3' untranslated regions--which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the PBLUESCRIPT phagemid (Stratagene, La Jolla, Calif.) or PSPORT1 plasmid (Gibco BRL, Gaithersburg, MD) and the like may be used. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are generally preferred. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding a polypeptide, vectors based on SV40 or EBV may be advantageously used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected

depending upon the use intended for the expressed polypeptide. For example, when large quantities are needed, for example for the induetion of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene), in which the sequence encoding the polypeptide of interest may be ligated into the vector in frame with sequenees for the amino-terminal Met and the subsequent 7 residues of .beta.-galactosidase so that a hybrid protein is produced; plN vectors (Van Heeke, G. and S. M. Schuster (1989) *J. Biol. Chem. 264*:5503-5509); and the like. pGEX Vectors (Promega, Madison, Wis.) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast, Saccharomyces cerevisiae, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. For reviews, see Ausubel et al. (supra) and Grant et al. (1987) *Methods Enzymol.* 153:516-544.

In cases where plant expression vectors are used, the expression of sequences encoding polypeptides may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) *EMBO J. 6*:307-311. Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used (Coruzzi, G. et al. (1984) *EMBO J. 3*:1671-1680; Broglie, R. et al. (1984) *Science 224*:838-843; and Winter, J. et al. (1991) *Results Probl. Cell Differ. 17*:85-105). These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (see, for example, Hobbs, S. or Murry, L. E. in McGraw Hill Yearbook of Science and Teehnology (1992) McGraw Hill, New York, N.Y.; pp. 191-196).

An insect system may also be used to express a polypeptide of interest. For example, in one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in Spodoptera frugiperda cells or in Trichoplusia larvae. The sequences encoding the polypeptide may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of the polypeptide-encoding sequence will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, S. frugiperda cells or Trichoplusia larvae in which the polypeptide of interest may be expressed (Engelhard, E. K. et al. (1994) *Proc. Natl. Acad. Sci. 91* :3224-3227).

In mammalian host cells, a number of viral-based expression systems are generally available. For example, in cases where an adenovirus is used as an expression vector, sequences encoding a polypeptide of interest may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing the polypeptide in infected host cells (Logan, J. and Shenk, T. (1984) *Proc. Natl. Acad. Sci. 81*:3655-3659). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be used to achieve more efficient translation of sequences encoding a polypeptide of interest. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding the polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used, such as those described in the literature (Scharf, D. et al. (1994) *Results Probl. Cell Differ. 20*:125-162).

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation. glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, HEK293, and WI38, which have specific cellular machinery and characteristic mechanisms for such post-translational activities, may be chosen to ensure the correct modification and processing of the foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is generally preferred. For example, cell lines which stably express a polynucleotide of interest may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler, M. et al. (1977) *Cell 11*:223-32) and adenine phosphoribosyltransferase (Lowy, I. et al. (1990) *Cell 22*:817-23) genes which can be employed in tk.sup.- or aprt.sup.- cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler, M. et al. (1980) *Proc. Natl. Acad. Sci. 77*:3567-70); npt, which confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin, F. et al (1981) *J. Mol. Biol. 150*:1-14); and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, *supra*). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman, S. C. and R. C. Mulligan (1988) *Proc. Natl. Acad. Sci.*

85:8047-51). Recently, the use of visible markers has gained popularity with such markers as anthocyanins, beta-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C. A. et al. (1995) *Methods Mol. Biol. 55*:121-131).

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For example, if the sequence encoding a polypeptide is inserted within a marker gene sequence, recombinant cells containing sequences can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a polypeptide-encoding sequence under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

Alternatively, host cells which contain and express a desired polynucleotide sequence may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques whieh include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein.

A variety of protocols for detecting and measuring the expression of polynucleotide-encoded products, using either polyclonal or monoclonal antibodies specific for the product are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on a given polypeptide may be preferred for some applications, but a competitive binding assay may also be employed. These and other assays are described, among other places, in Hampton, R. et al. (1990; Serological Methods, a Laboratory Manual, APS Press, St Paul. Minn.) and Maddox, D. E. et al. (1983; *J. Exp. Med. 158*:1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to

polynucleotides include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the sequences, or any portions thereof may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits. Suitable reporter molecules or labels, which may be used include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host eells transformed with a polynucleotide sequence of interest may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides of the invention may be designed to contain signal sequenees which direct secretion of the encoded polypeptide through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may be used to join sequences encoding a polypeptide of interest to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). The inclusion of cleavable linker sequences such as those specifie for Factor XA or enterokinase (Invitrogen. San Diego, Calif.) between the purification domain and the encoded polypeptide may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a polypeptide of interest and a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography) as described in Porath, J. et al. (1992, *Prot. Exp. Purif. 3*:263-281) while the enterokinase cleavage site provides a means for purifying the desired polypeptide from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D. J.

et al. (1993; *DNA Cell Biol. 12*:441-453).

In addition to recombinant production methods, polypeptides of the invention, and fragments thereof, may be produced by direct peptide synthesis using solid-phase techniques (Merrifield J. (1963) *J. Am. Chem. Soc. 85*:2149-2154). Protein synthesis may be performed using manual teehniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Alternatively, various fragments may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

SITE-SPECIFIC MUTAGENESIS

Site-specific mutagenesis is a technique useful in the preparation of individual peptides, or biologically functional equivalent polypeptides, through specifie mutagenesis of the underlying polynucleotides that encode them. The technique, well-known to those of skill in the art, further provides a ready ability to prepare and test sequence variants, for example, incorporating one or more of the foregoing considerations, by introducing one or more nucleotide sequence changes into the DNA. Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Mutations may be employed in a selected polynucleotide sequence to improve, alter, decrease, modify, or otherwise change the properties of the polynucleotide itself, and/or alter the properties, activity, composition, stability, or primary sequence of the encoded polypeptide.

In certain embodiments of the present invention, the inventors contemplate the mutagenesis of the disclosed polynucleotide sequences to alter one or more properties of the encoded polypeptide, such as the antigenicity of a polypeptide vaccine. The techniques of site-specifie mutagenesis are well-known in the art, and are widely used to create variants of both polypeptides and polynucleotides. For example, site-specific mutagenesis is often used to alter a specific portion of a DNA molecule. In such embodiments, a primer comprising typically about 14 to about 25 nucleotides or so

in length is employed, with about 5 to about 10 residues on both sides of the junction of the sequence being altered.

As will be appreciated by those of skill in the art, site-specific mutagenesis techniques have often employed a phage vector that exists in both a single stranded and double stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage. These phage are readily commercially-available and their use is generally well-known to those skilled in the art. Double-stranded plasmids are also routinely employed in site directed mutagenesis that eliminates the step of transferring the gene of interest from a plasmid to a phage.

In general, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector or melting apart of two strands of a double-stranded vector that includes within its sequence a DNA sequence that encodes the desired peptide. An oligonucleotide primer bearing the desired mutated sequence is prepared, generally synthetically. This primer is then annealed with the single-stranded vector, and subjected to DNA polymerizing enzymes such as *E. coli* polymerase I Klenow fragment, in order to complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells, such as *E. coli* cells, and clones are selected which include recombinant vectors bearing the mutated sequence arrangement.

The preparation of sequence variants of the selected peptide-encoding DNA segments using site-directed mutagenesis provides a means of producing potentially useful species and is not meant to be limiting as there are other ways in which sequence variants of peptides and the DNA sequences encoding them may be obtained. For example, recombinant vectors encoding the desired peptide sequence may be treated with mutagenie agents, such as hydroxylamine, to obtain sequence variants. Specific details regarding these methods and protocols are found in the teachings of Maloy *et al.*, 1994; Segal, 1976; Prokop and Bajpai, 1991; Kuby, 1994; and Maniatis *et al.*, 1982, each incorporated herein by reference, for that purpose.

As used herein, the term "oligonucleotide directed mutagenesis procedure" refers to template-dependent processes and vector-mediated propagation which result in an inerease in the concentration of a specific nucleic acid molecule

relative to its initial concentration, or in an increase in the concentration of a detectable signal, such as amplification. As used herein, the term "oligonucleotide directed mutagenesis procedure" is intended to refer to a process that involves the template-dependent extension of a primer molecule. The term template dependent process refers to nucleic acid synthesis of an RNA or a DNA moleeule wherein the sequence of the newly synthesized strand of nucleic acid is dictated by the well-known rules of complementary base pairing (see, for example, Watson, 1987). Typically, vector mediated methodologies involve the introduction of the nucleic acid fragment into a DNA or RNA vector, the clonal amplification of the vector, and the recovery of the amplified nucleic acid fragment. Examples of such methodologies are provided by U. S. Patent No. 4,237,224, specifically incorporated herein by reference in its entirety.

POLYNUCLEOTIDE AMPLIFICATION TECHNIQUES

A number of template dependent processes are available to amplify the target sequences of interest present in a sample. One of the best known amplification methods is the polymerase chain reaction (PCR™) which is described in detail in U.S. Patent Nos. 4,683,195, 4,683,202 and 4,800,159, each of which is incorporated herein by reference in its entirety. Briefly, in PCR™, two primer sequences are prepared which are complementary to regions on opposite complementary strands of the target sequence. An excess of deoxynucleoside triphosphates is added to a reaction mixture along with a DNA polymerase (e.g., *Taq* polymerase). If the target sequence is present in a sample, the primers will bind to the target and the polymerase will cause the primers to be extended along the target sequence by adding on nucleotides. By raising and lowering the temperature of the reaction mixture, the extended primers will dissociate from the target to form reaction products, exeess primers will bind to the target and to the reaction product and the process is repeated. Preferably reverse transcription and PCR™ amplification procedure may be performed in order to quantify the amount of mRNA amplified. Polymerase chain reaction methodologies are well known in the art.

Another method for amplification is the ligase chain reaction (referred to as LCR), disclosed in Eur. Pat. Appl. Publ. No. 320,308 (specifically incorporated herein by reference in its entirety). In LCR, two complementary probe pairs are

prepared, and in the presence of the target sequence, each pair will bind to opposite complementary strands of the target such that they abut. In the presence of a ligase, the two probe pairs will link to form a single unit. By temperature cycling, as in PCR™, bound ligated units dissociate from the target and then serve as "target sequences" for ligation of excess probe pairs. U.S. Patent No. 4,883,750, incorporated herein by reference in its entirety, describes an alternative method of amplification similar to LCR for binding probe pairs to a target sequence.

Qbeta Replicase, described in PCT Intl. Pat. Appl. Publ. No. PCT/US87/00880, incorporated herein by reference in its entirety, may also be used as still another amplification method in the present invention. In this method, a replicative sequence of RNA that has a region complementary to that of a target is added to a sample in the presence of an RNA polymerase. The polymerase will copy the replicative sequence that can then be detected.

An isothermal amplification method, in which restriction endonueleases and ligases are used to achieve the amplification of target molecules that contain nucleotide 5'-[α-thio]triphosphates in one strand of a restriction site (Walker *et al.*, 1992, incorporated herein by reference in its entirety), may also be useful in the amplification of nucleic acids in the present invention.

Strand Displacement Amplification (SDA) is another method of carrying out isothermal amplification of nucleic acids which involves multiple rounds of strand displacement and synthesis, *i.e.* nick translation. A similar method, called Repair Chain Reaction (RCR) is another method of amplification which may be useful in the present invention and is involves annealing several probes throughout a region targeted for amplification, followed by a repair reaction in which only two of the four bases are present. The other two bases can be added as biotinylated derivatives for easy detection. A similar approach is used in SDA.

Sequences can also be detected using a cyclic probe reaction (CPR). In CPR, a probe having a 3' and 5' sequences of non-target DNA and an internal or "middle" sequence of the target protein specific RNA is hybridized to DNA which is present in a sample. Upon hybridization, the reaction is treated with RNaseH, and the products of the probe are identified as distinctive products by generating a signal that is released after digestion. The original template is annealed to another cyeling probe and

the reaction is repeated. Thus, CPR involves amplifying a signal generated by hybridization of a probe to a target gene specific expressed nucleic acid.

Still other amplification methods described in Great Britain Pat. Appl. No. 2 202 328, and in PCT Intl. Pat. Appl. Publ. No. PCT/US89/01025, each of which is incorporated herein by reference in its entirety, may be used in accordance with the present invention. In the former application, "modified" primers are used in a PCR-like, template and enzyme dependent synthesis. The primers may be modified by labeling with a capture moiety (*e.g.,* biotin) and/or a detector moiety (*e.g.,* enzyme). In the latter application, an excess of labeled probes is added to a sample. In the presence of the target sequence, the probe binds and is cleaved catalytically. After cleavage, the target sequence is released intact to be bound by excess probe. Cleavage of the labeled probe signals the presence of the target sequence.

Other nucleic acid amplification procedures include transcription-based amplification systems (TAS) (Kwoh *et al.,* 1989; PCT Intl. Pat. Appl. Publ. No. WO 88/10315, incorporated herein by reference in its entirety), including nucleic acid sequence based amplification (NASBA) and 3SR. In NASBA, the nucleic acids can be prepared for amplification by standard phenol/chloroform extraction, heat denaturation of a sample, treatment with lysis buffer and minispin columns for isolation of DNA and RNA or guanidinium chloride extraction of RNA. These amplification techniques involve annealing a primer that has sequences specific to the target sequence. Following polymerization, DNA/RNA hybrids are digested with RNase H while double stranded DNA molecules are heat-denatured again. In either case the single stranded DNA is made fully double stranded by addition of second target-specific primer, followed by polymerization. The double stranded DNA molecules are then multiply transcribed by a polymerase such as T7 or SP6. In an isothermal cyclic reaction, the RNAs are reverse transcribed into DNA, and transcribed once again with a polymerase such as T7 or SP6. The resulting products, whether truncated or complete, indicate target-specific sequences.

Eur. Pat. Appl. Publ. No. 329,822, incorporated herein by reference in its entirety, disclose a nucleic acid amplification process involving cyclically synthesizing single-stranded RNA ("ssRNA"), ssDNA, and double-stranded DNA (dsDNA), which may be used in accordance with the present invention. The ssRNA is a first template

for a first primer oligonucleotide, which is elongated by reverse transcriptase (RNA-dependent DNA polymerase). The RNA is then removed from resulting DNA:RNA duplex by the action of ribonuclease H (RNase H, an RNase specific for RNA in a duplex with either DNA or RNA). The resultant ssDNA is a second template for a second primer, which also includes the sequences of an RNA polymerase promoter (exemplified by T7 RNA polymerase) 5' to its homology to its template. This primer is then extended by DNA polymerase (exemplified by the large "Klenow" fragment of *E. coli* DNA polymerase I), resulting as a double-stranded DNA ("dsDNA") molecule, having a sequence identical to that of the original RNA between the primers and having additionally, at one end, a promoter sequence. This promoter sequence can be used by the appropriate RNA polymerase to make many RNA copies of the DNA. These copies can then re-enter the cycle leading to very swift amplification. With proper choice of enzymes, this amplification can be done isothermally without addition of enzymes at each cycle. Because of the cyclical nature of this process, the starting sequence can be chosen to be in the form of either DNA or RNA.

PCT Intl. Pat. Appl. Publ. No. WO 89/06700, incorporated herein by reference in its entirety, disclose a nucleic acid sequence amplification scheme based on the hybridization of a promoter/primer sequence to a target single-stranded DNA ("ssDNA") followed by transcription of many RNA copies of the sequence. This scheme is not cyclic; i.e. new templates are not produced from the resultant RNA transcripts. Other amplification methods include "RACE" (Frohman, 1990), and "one-sided PCR" (Ohara, 1989) which are well-known to those of skill in the art.

Methods based on ligation of two (or more) oligonucleotides in the presenee of nucleic aeid having the sequence of the resulting "di-oligonucleotide", thereby amplifying the di-oligonucleotide (Wu and Dean, 1996, incorporated herein by reference in its entirety), may also be used in the amplification of DNA sequences of the present invention.

BIOLOGICAL FUNCTIONAL EQUIVALENTS

Modification and changes may be made in the structure of the polynucleotides and polypeptides of the present invention and still obtain a functional molecule that encodes a polypeptide with desirable characteristics. As mentioned

above, it is often desirable to introduce one or more mutations into a specific polynucleotide sequence. In certain circumstances, the resulting encoded polypeptide sequence is altered by this mutation, or in other cases, the sequence of the polypeptide is unchanged by one or more mutations in the encoding polynucleotide.

When it is desirable to alter the amino acid sequence of a polypeptide to create an equivalent, or even an improved, second-generation moleeule, the amino acid changes may be achieved by changing one or more of the codons of the encoding DNA sequence, according to Table 1.

For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence, and, of course, its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. It is thus contemplated by the inventors that various changes may be made in the peptide sequences of the disclosed eompositions, or corresponding DNA sequences which encode said peptides without appreciable loss of their biological utility or activity.

TABLE 1

Amino Acids			Codons					
Alanine	Ala	A	GCA	GCC	GCG	GCU		
Cysteine	Cys	C	UGC	UGU				
Aspartic acid	Asp	D	GAC	GAU				
Glutamic acid	Glu	E	GAA	GAG				
Phenylalanine	Phe	F	UUC	UUU				
Glycine	Gly	G	GGA	GGC	GGG	GGU		
Histidine	His	H	CAC	CAU				
Isoleucine	Ile	I	AUA	AUC	AUU			
Lysine	Lys	K	AAA	AAG				
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU
Methionine	Met	M	AUG					
Asparagine	Asn	N	AAC	AAU				
Proline	Pro	P	CCA	CCC	CCG	CCU		
Glutamine	Gln	Q	CAA	CAG				
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGU
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU
Threonine	Thr	T	ACA	ACC	ACG	ACU		
Valine	Val	V	GUA	GUC	GUG	GUU		
Tryptophan	Trp	W	UGG					
Tyrosine	Tyr	Y	UAC	UAU				

In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982, incorporated herein by reference). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics (Kyte and Doolittle, 1982). These values are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (–0.4); threonine (–0.7); serine (–0.8);

tryptophan (–0.9); tyrosine (–1.3); proline (–1.6); histidine (–3.2); glutamate (–3.5); glutamine (–3.5); aspartate (–3.5); asparagine (–3.5); lysine (–3.9); and arginine (–4.5).

It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathie index or score and still result in a protein with similar biological activity, *i.e.* still obtain a biological functionally equivalent protein. In making sueh changes, the substitution of amino acids whose hydropathic indices are within ±2 is preferred, those within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred. It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U. S. Patent 4,554,101 (specifically ineorporated herein by referenee in its entirety), states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, eorrelates with a biological property of the protein.

As detailed in U. S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (–0.4); proline (–0.5 ± 1); alanine (–0.5); histidine (–0.5); cysteine (–1.0); methionine (–1.3); valine (–1.5); leucine (–1.8); isoleucine (–1.8); tyrosine (–2.3); phenylalanine (–2.5); tryptophan (–3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various of the foregoing characteristies into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

In addition, any polynucleotide may be further modified to increase stability *in vivo*. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl

rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine and wybutosine, as well as acetyl-methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

IN VIVO POLYNUCLEOTIDE DELIVERY TECHNIQUES

In additional embodiments, genetic constructs comprising one or more of the polynucleotides of the invention are introduced into cells *in vivo*. This may be achieved using any of a variety or well known approaches, several of which are outlined below for the purpose of illustration.

1. ADENOVIRUS

One of the preferred methods for *in vivo* delivery of one or more nucleic acid sequences involves the use of an adenovirus expression vector. "Adenovirus expression vector" is meant to include those constructs containing adenovirus sequences sufficient to (a) support packaging of the construct and (b) to express a polynucleotide that has been cloned therein in a sense or antisense orientation. Of course, in the context of an antisense construct, expression does not require that the gene product be synthesized.

The expression vector comprises a genetically engineered form of an adenovirus. Knowledge of the genetic organization of adenovirus, a 36 kb, linear, double-stranded DNA virus, allows substitution of large pieces of adenoviral DNA with foreign sequences up to 7 kb (Grunhaus and Horwitz, 1992). In contrast to retrovirus, the adenoviral infection of host cells does not result in chromosomal integration because adenoviral DNA can replicate in an episomal manner without potential genotoxicity. Also, adenoviruses are structurally stable, and no genome rearrangement has been detected after extensive amplification. Adenovirus can infect virtually all epithelial cells regardless of their cell cycle stage. So far, adenoviral infection appears to be linked only to mild disease such as acute respiratory disease in humans.

Adenovirus is particularly suitable for use as a gene transfer vector because of its mid-sized genome, ease of manipulation, high titer, wide target-cell range and high infectivity. Both ends of the viral genome contain 100-200 base pair inverted repeats (ITRs), which are *cis* elements necessary for viral DNA replication and

packaging. The early (E) and late (L) regions of the genome eontain different transcription units that are divided by the onset of viral DNA replication. The E1 region (E1A and E1B) encodes proteins responsible for the regulation of transcription of the viral genome and a few cellular genes. The expression of the E2 region (E2A and E2B) results in the synthesis of the proteins for viral DNA replication. These proteins are involved in DNA replication, late gene expression and host cell shut-off (Renan, 1990). The products of the late genes, including the majority of the viral capsid proteins, are expressed only after significant processing of a single primary transcript issued by the major late promoter (MLP). The MLP, (located at 16.8 m.u.) is partieularly efficient during the late phase of infection, and all the mRNA's issued from this promoter possess a 5′-tripartite leader (TPL) sequence which makes them preferred mRNA's for translation.

In a current system, recombinant adenovirus is generated from homologous recombination between shuttle vector and provirus vector. Due to the possible recombination between two proviral vectors, wild-type adenovirus may be generated from this process. Therefore, it is critical to isolate a single clone of virus from an individual plaque and examine its genomic structure.

Generation and propagation of the current adenovirus vectors, which are replication deficient, depend on a unique helper cell line, designated 293, which was transformed from human embryonic kidney cells by Ad5 DNA fragments and constitutively expresses E1 proteins (Graham *et al.*, 1977). Since the E3 region is dispensable from the adenovirus genome (Jones and Shenk, 1978), the current adenovirus veetors, with the help of 293 cells, carry foreign DNA in either the E1, the D3 or both regions (Graham and Prevec, 1991). In nature, adenovirus ean package approximately 105% of the wild-type genome (Ghosh-Choudhury *et al.*, 1987), providing capacity for about 2 extra kB of DNA. Combined with the approximately 5.5 kB of DNA that is replaceable in the E1 and E3 regions, the maximum capaeity of the current adenovirus vector is under 7.5 kB, or about 15% of the total length of the vector. More than 80% of the adenovirus viral genome remains in the vector backbone and is the source of vector-borne cytotoxicity. Also, the replication deficiency of the E1-deleted virus is incomplete. For example, leakage of viral gene expression has been

observed with the currently available vectors at high multiplicities of infection (MOI) (Mulligan, 1993).

Helper cell lines may be derived from human cells such as human embryonic kidney cells, muscle cells, hematopoietic cells or other human embryonic mesenchymal or epithelial cells. Alternatively, the helper cells may be derived from the cells of other mammalian species that are permissive for human adenovirus. Such cells include, *e.g.*, Vero cells or other monkey embryonic mesenchymal or epithelial cells. As stated above, the currently preferred helper cell line is 293.

Recently, Racher *et al.* (1995) disclosed improved methods for culturing 293 cells and propagating adenovirus. In one format, natural cell aggregates are grown by inoculating individual cells into 1 liter siliconized spinner flasks (Techne, Cambridge, UK) containing 100-200 ml of medium. Following stirring at 40 rpm, the cell viability is estimated with trypan blue. In another format, Fibra-Cel microcarriers (Bibby Sterlin, Stone, UK) (5 g/l) is employed as follows. A cell inoculum, resuspended in 5 ml of medium, is added to the carrier (50 ml) in a 250 ml Erlenmeyer flask and left stationary, with occasional agitation, for 1 to 4 h. The medium is then replaced with 50 ml of fresh medium and shaking initiated. For virus production, cells are allowed to grow to about 80% confluence, after which time the medium is replaced (to 25% of the final volume) and adenovirus added at an MOI of 0.05. Cultures are left stationary overnight, following which the volume is increased to 100% and shaking commenced for another 72 h.

Other than the requirement that the adenovirus vector be replication defective, or at least conditionally defective, the nature of the adenovirus vector is not believed to be crucial to the successful practice of the invention. The adenovirus may be of any of the 42 different known serotypes or subgroups A-F. Adenovirus type 5 of subgroup C is the preferred starting material in order to obtain a conditional replication-defective adenovirus vector for use in the present invention, since Adenovirus type 5 is a human adenovirus about which a great deal of biochemical and genetic information is known, and it has historically been used for most constructions employing adenovirus as a vector.

As stated above, the typical vector according to the present invention is replication defective and will not have an adenovirus E1 region. Thus, it will be most

convenient to introduce the polynucleotide encoding the gene of interest at the position from which the E1-coding sequences have been removed. However, the position of insertion of the construct within the adenovirus sequences is not critical to the invention. The polynucleotide encoding the gene of interest may also be inserted in lieu of the deleted E3 region in E3 replacement vectors as described by Karlsson *et al.* (1986) or in the E4 region where a helper cell line or helper virus complements the E4 defect.

Adenovirus is easy to grow and manipulate and exhibits broad host range *in vitro* and *in vivo*. This group of viruses can be obtained in high titers, *e.g.*, 10^9-10^{11} plaque-forming units per ml, and they are highly infective. The life cycle of adenovirus does not require integration into the host cell genome. The foreign genes delivered by adenovirus vectors are episomal and, therefore, have low genotoxicity to host cells. No side effects have been reported in studies of vaccination with wild-type adenovirus (Couch *et al.*, 1963; Top *et al.*, 1971), demonstrating their safety and therapeutic potential as *in vivo* gene transfer veetors.

Adenovirus vectors have been used in eukaryotic gene expression (Levrero *et al.*, 1991; Gomez-Foix *et al.*, 1992) and vaccine development (Grunhaus and Horwitz, 1992; Graham and Prevec, 1992). Recently, animal studies suggested that recombinant adenovirus could be used for gene therapy (Stratford-Perricaudet and Perrieaudet, 1991; Stratford-Perricaudet *et al.*, 1990; Rich *et al.*, 1993). Studies in administering recombinant adenovirus to different tissues include trachea instillation (Rosenfeld *et al.*, 1991; Rosenfeld *et al.*, 1992), muscle injection (Ragot *et al.*, 1993), peripheral intravenous injections (Herz and Gerard, 1993) and stereotactic inoculation into the brain (Le Gal La Salle *et al.*, 1993).

2. RETROVIRUSES

The retroviruses are a group of single-stranded RNA viruses characterized by an ability to convert their RNA to double-stranded DNA in infected eells by a process of reverse-transcription (Coffin, 1990). The resulting DNA then stably integrates into cellular chromosomes as a provirus and directs synthesis of viral proteins. The integration results in the retention of the viral gene sequences in the recipient cell and its descendants. The retroviral genome contains three genes, gag, pol, and env that code for capsid proteins, polymerase enzyme, and envelope components,

respectively. A sequence found upstream from the gag gene contains a signal for paekaging of the genome into virions. Two long terminal repeat (LTR) sequences are present at the 5′ and 3′ ends of the viral genome. These contain strong promoter and enhancer sequences and are also required for integration in the host cell genome (Coffin, 1990).

In order to construct a retroviral vector, a nucleic acid encoding one or more oligonucleotide or polynucleotide sequences of interest is inserted into the viral genome in the place of certain viral sequences to produce a virus that is replication-defeetive. In order to produce virions, a packaging cell line containing the gag, pol, and env genes but without the LTR and packaging components is constructed (Mann *et al.*, 1983). When a recombinant plasmid containing a cDNA, together with the retroviral LTR and packaging sequences is introduced into this cell line (by caleium phosphate precipitation for example), the packaging sequence allows the RNA transcript of the recombinant plasmid to be packaged into viral particles, which are then secreted into the culture media (Nicolas and Rubenstein, 1988; Temin, 1986; Mann *et al.*, 1983). The media containing the recombinant retroviruses is then eollected, optionally concentrated, and used for gene transfer. Retroviral vectors are able to infect a broad variety of cell types. However, integration and stable expression require the division of host cells (Paskind *et al.*, 1975).

A novel approach designed to allow specific targeting of retrovirus vectors was recently developed based on the chemical modification of a retrovirus by the chemical addition of lactose residues to the viral envelope. This modification could permit the specific infection of hepatocytes *via* sialoglycoprotein receptors.

A different approach to targeting of recombinant retroviruses was designed in which biotinylated antibodies against a retroviral envelope protein and against a specific cell receptor were used. The antibodies were coupled *via* the biotin components by using streptavidin (Roux *et al.*, 1989). Using antibodies against major histocompatibility complex class I and class II antigens, they demonstrated the infection of a variety of human cells that bore those surface antigens with an ecotropic virus *in vitro* (Roux *et al.*, 1989).

3. ADENO-ASSOCIATED VIRUSES

AAV (Ridgeway, 1988; Hermonat and Muzyczka, 1984) is a parovirus, discovered as a contamination of adenoviral stocks. It is a ubiquitous virus (antibodies are present in 85% of the US human population) that has not been linked to any disease. It is also classified as a dependovirus, because its replications is dependent on the presence of a helper virus, such as adenovirus. Five serotypes have been isolated, of which AAV-2 is the best characterized. AAV has a single-stranded linear DNA that is encapsidated into capsid proteins VP1, VP2 and VP3 to form an icosahedral virion of 20 to 24 nm in diameter (Muzyczka and McLaughlin, 1988).

The AAV DNA is approximately 4.7 kilobases long. It contains two open reading frames and is flanked by two ITRs (FIG. 2). There are two major genes in the AAV genome: *rep* and *cap*. The *rep* gene codes for proteins responsible for viral replications, whereas *cap* codes for capsid protein VP1-3. Each ITR forms a T-shaped hairpin structure. These terminal repeats are the only essential *cis* components of the AAV for chromosomal integration. Therefore, the AAV can be used as a vector with all viral coding sequences removed and replaced by the cassette of genes for delivery. Three viral promoters have been identified and named p5, p19, and p40, according to their map position. Transcription from p5 and p19 results in production of rep proteins, and transcription from p40 produces the capsid proteins (Hermonat and Muzyczka, 1984).

There are several factors that prompted researchers to study the possibility of using rAAV as an expression vector One is that the requirements for delivering a gene to integrate into the host chromosome are surprisingly few. It is necessary to have the 145-bp ITRs, which are only 6% of the AAV genome. This leaves room in the vector to assemble a 4.5-kb DNA insertion. While this carrying capacity may prevent the AAV from delivering large genes, it is amply suited for delivering the antisense constructs of the present invention.

AAV is also a good choice of delivery vehicles due to its safety. There is a relatively complicated rescue mechanism: not only wild type adenovirus but also AAV genes are required to mobilize rAAV. Likewise, AAV is not pathogenic and not associated with any disease. The removal of viral coding sequences minimizes immune

reactions to viral gene expression, and therefore, rAAV does not evoke an inflammatory response.

4. OTHER VIRAL VECTORS AS EXPRESSION CONSTRUCTS

Other viral vectors may be employed as expression constructs in the present invention for the delivery of oligonucleotide or polynucleotide sequences to a host cell. Vectors derived from viruses such as vaceinia virus (Ridgeway, 1988; Coupar *et al.*, 1988), lentiviruses, polio viruses and herpes viruses may be employed. They offer several attractive features for various mammalian cells (Friedmann, 1989; Ridgeway, 1988; Coupar *et al.*, 1988; Horwich *et al.*, 1990).

With the recent recognition of defective hepatitis B viruses, new insight was gained into the structure-function relationship of different viral sequences. *In vitro* studies showed that the virus could retain the ability for helper-dependent packaging and reverse transcription despite the deletion of up to 80% of its genome (Horwich *et al.*, 1990). This suggested that large portions of the genome eould be replaced with foreign genetic material. The hepatotropism and persistencc (integration) were particularly attractive properties for liver-directed gene transfer. Chang *et al.* (1991) introduced the chloramphenicol acetyltransferase (CAT) gene into duck hepatitis B virus genome in the place of the polymerase, surface, and pre-surface coding sequences. It was cotransfected with wild-type virus into an avian hepatoma cell line. Culture media eontaining high titers of the recombinant virus were used to infect primary duckling hepatocytes. Stable CAT gene expression was detected for at least 24 days after transfection (Chang *et al.*, 1991).

5. NON-VIRAL VECTORS

In order to effect expression of the oligonucleotide or polynucleotide sequences of the present invention, the expression construct must be delivered into a cell. This delivery may be accomplished *in vitro*, as in laboratory procedures for transforming cells lines, or *in vivo* or *ex vivo*, as in the treatment of certain disease states. As described above, one preferred mechanism for delivery is *via* viral infection where the expression construct is encapsulated in an infectious viral particle.

Once the expression construct has been delivered into the eell the nucleic acid encoding the desired oligonucleotide or polynucleotide sequences may be positioned and expressed at different sites. In certain embodiments, the nucleic acid

encoding the construct may be stably integrated into the genome of the cell. This integration may be in the specific location and orientation *via* homologous recombination (gene replacement) or it may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the nucleic acid may be stably maintained in the cell as a separate, episomal segment of DNA. Such nucleic acid segments or "episomes" encode sequences sufficient to permit maintenance and replication independent of or in synchronization with the host cell cycle. How the expression construct is delivered to a cell and where in the cell the nucleic acid remains is dependent on the type of expression construct employed.

In certain embodiments of the invention, the expression construct comprising one or more oligonucleotide or polynucleotide sequences may simply consist of naked recombinant DNA or plasmids. Transfer of the construct may be performed by any of the methods mentioned above which physically or chemically permeabilize the cell membrane. This is particularly applicable for transfer *in vitro* but it may be applied to *in vivo* use as well. Dubensky *et al.* (1984) successfully injected polyomavirus DNA in the form of calcium phosphate precipitates into liver and spleen of adult and newborn mice demonstrating active viral replication and acute infection. Benvenisty and Reshef (1986) also demonstrated that direct intraperitoneal injection of calcium phosphate-precipitated plasmids results in expression of the transfected genes. It is envisioned that DNA encoding a gene of interest may also be transferred in a similar manner *in vivo* and express the gene product.

Another embodiment of the invention for transferring a naked DNA expression construct into cells may involve particle bombardment. This method depends on the ability to accelerate DNA-coated microprojectiles to a high velocity allowing them to pierce cell membranes and enter cells without killing them (Klein *et al.*, 1987). Several devices for accelerating small particles have been developed. One such device relies on a high voltage discharge to generate an electrical current, which in turn provides the motive force (Yang *et al.*, 1990). The microprojectiles used have consisted of biologically inert substances such as tungsten or gold beads.

Selected organs including the liver, skin, and muscle tissue of rats and mice have been bombarded *in vivo* (Yang *et al.*, 1990; Zelenin *et al.*, 1991). This may require surgical exposure of the tissue or cells, to eliminate any intervening tissue

between the gun and the target organ, *i.e. ex vivo* treatment. Again, DNA encoding a particular gene may be delivered *via* this method and still be incorporated by the present invention.

ANTISENSE OLIGONUCLEOTIDES

The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand exeept that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

The targeting of antisense oligonucleotides to mRNA is thus one mechanism to shut down protein synthesis, and, consequently, represents a powerful and targeted therapeutic approach. For example, the synthesis of polygalactauronase and the muscarine type 2 aeetylcholine receptor are inhibited by antisense oligonucleotides directed to their respective mRNA sequences (U. S. Patent 5,739,119 and U. S. Patent 5,759,829, each specifically incorporated herein by reference in its entirety). Further, examples of antisense inhibition have been demonstrated with the nuclear protein cyclin, the multiple drug resistance gene (MDG1), ICAM-1, E-selectin, STK-1, striatal $GABA_A$ receptor and human EGF (Jaskulski *et al.*, 1988; Vasanthakumar and Ahmed, 1989; Peris *et al.*, 1998; U. S. Patent 5,801,154; U. S. Patent 5,789,573; U. S. Patent 5,718,709 and U. S. Patent 5,610,288, each specifically incorporated herein by reference in its entirety). Antisense constructs have also been described that inhibit and can be used to treat a variety of abnormal cellular proliferations, *e.g.* caneer (U. S. Patent 5,747,470; U. S. Patent 5,591,317 and U. S. Patent 5,783,683, each speeifically incorporated herein by reference in its entirety).

Therefore, in exemplary embodiments, the invention provides oligonucleotide sequences that comprise all, or a portion of, any sequence that is

capable of specifically binding to polynucleotide sequence described herein, or a complement thereof. In one embodiment, the antisense oligonucleotides comprise DNA or derivatives thereof. In another embodiment, the oligonueleotides comprise RNA or derivatives thereof. In a third embodiment, the oligonucleotides are modified DNAs comprising a phosphorothioated modified backbone. In a fourth embodiment, the oligonucleotide sequences comprise peptide nucleic acids or derivatives thereof. In each case, preferred compositions comprise a sequence region that is complementary, and more preferably substantially-complementary, and even more preferably, completely complementary to one or more portions of polynucleotides disclosed herein.

Selection of antisense compositions specific for a given gene sequence is based upon analysis of the ehosen target sequence (*i.e.* in these illustrative examples the rat and human sequences) and determination of secondary structure, T_m, binding energy, relative stability, and antisense compositions were selected based upon their relative inability to form dimers, hairpins, or other secondary structures that would reduce or prohibit specific binding to the target mRNA in a host cell.

Highly preferred target regions of the mRNA, are those which are at or near the AUG translation initiation codon, and those sequences whieh were substantially complementary to 5' regions of the mRNA. These secondary structure analyses and target site selection considerations were performed using v.4 of the OLIGO primer analysis software (Rychlik, 1997) and the BLASTN 2.0.5 algorithm software (Altschul *et al.,* 1997).

The use of an antisense delivery method employing a short peptide vector, termed MPG (27 residues), is also contemplated. The MPG peptide contains a hydrophobic domain derived from the fusion sequence of HIV gp41 and a hydrophilic domain from the nuclear localization sequenee of SV40 T-antigen (Morris *et al.*, 1997). It has been demonstrated that several molecules of the MPG peptide coat the antisense oligonucleotides and ean be delivered into cultured mammalian cells in less than 1 hour with relatively high efficiency (90%). Further, the interaction with MPG strongly increases both the stability of the oligonucleotide to nuclease and the ability to cross the plasma membrane (Morris *et al.*, 1997).

RIBOZYMES

Although proteins traditionally have been used for catalysis of nucleic acids, another class of macromolecules has emerged as useful in this endeavor. Ribozymes are RNA-protein complexes that cleave nucleic acids in a site-specific fashion. Ribozymes have specific catalytic domains that possess endonuclcase activity (Kim and Cech, 1987; Gerlach *et al.*, 1987; Forster and Symons, 1987). For example, a large number of ribozymes accelerate phosphoester transfer reactions with a high degree of specificity, often cleaving only one of several phosphoesters in an oligonucleotide substrate (Cech *et al.*, 1981; Michel and Westhof, 1990; Reinhold-Hurek and Shub, 1992). This specificity has been attributed to the requirement that the substrate bind via specific base-pairing interactions to the internal guide sequence ("IGS") of the ribozyme prior to chemical reaction.

Ribozyme catalysis has primarily been observed as part of sequence-specific cleavage/ligation reactions involving nucleic acids (Joyce, 1989; Cech *et al.*, 1981). For example, U. S. Patent No. 5,354,855 (specifically incorporated herein by reference) reports that certain ribozymes can act as endonucleases with a sequence specificity greater than that of known ribonueleases and approaching that of the DNA restriction enzymes. Thus, sequence-specific ribozyme-mediated inhibition of gene expression may be particularly suited to therapeutic applications (Scanlon *et al.*, 1991; Sarver *et al.*, 1990). Recently, it was reported that ribozymes elicited genetic changes in some cells lines to which they were applied; the altered genes included the oncogenes H-*ras*, c-*fos* and genes of HIV. Most of this work involved the modification of a target mRNA, based on a specific mutant codon that is cleaved by a specific ribozyme.

Six basic varieties of naturally-occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds *in trans* (and thus can cleave other RNA molecules) under physiological conditions. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to

direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

The enzymatic nature of a ribozyme is advantageous over many technologies, such as antisense technology (where a nucleic acid molecule simply binds to a nucleic acid target to block its translation) since the concentration of ribozyme necessary to affect a therapeutic treatment is lower than that of an antisense oligonucleotide. This advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can completely eliminate catalytic activity of a ribozyme. Similar mismatches in antisense molecules do not prevent their action (Woolf *et al.*, 1992). Thus, the specificity of action of a ribozyme is greater than that of an antisense oligonucleotide binding the same RNA site.

The enzymatic nucleic acid molecule may be formed in a hammerhead, hairpin, a hepatitis δ virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) or Neurospora VS RNA motif. Examples of hammerhead motifs are described by Rossi *et al.* (1992). Examples of hairpin motifs are described by Hampel *et al.* (Eur. Pat. Appl. Publ. No. EP 0360257), Hampel and Tritz (1989), Hampel *et al.* (1990) and U. S. Patent 5,631,359 (specifically incorporated herein by reference). An example of the hepatitis δ virus motif is described by Perrotta and Been (1992); an example of the RNaseP motif is described by Guerrier-Takada *et al.* (1983); Neurospora VS RNA ribozyme motif is described by Collins (Saville and Collins, 1990; Saville and Collins, 1991; Collins and Olive, 1993); and an example of the Group I intron is described in (U. S. Patent 4,987,071, specifically incorporated herein by reference). All that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule. Thus the ribozyme constructs need not be limited to specific motifs mentioned herein.

In certain embodiments, it may be important to produce enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of a desired target, such as one of the sequences disclosed herein. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target mRNA. Such enzymatic nucleic acid molecules can be delivered exogenously to specific cells as required. Alternatively, the ribozymes can be expressed from DNA or RNA vectors that are delivered to specific cells.

Small enzymatic nucleic acid motifs (*e.g.*, of the hammerhead or the hairpin structure) may also be used for exogenous delivery. The simple structure of these molecules increases the ability of the enzymatic nucleic acid to invade targeted regions of the mRNA structure. Alternatively, catalytic RNA molecules can be expressed within cells from eukaryotic promoters (*e.g.*, Scanlon *et al.*, 1991; Kashani-Sabet *et al.*, 1992; Dropulic *et al.*, 1992; Weerasinghe *et al.*, 1991; Ojwang *et al.*, 1992; Chen *et al.*, 1992; Sarver *et al.*, 1990). Those skilled in the art realize that any ribozyme can be expressed in eukaryotic cells from the appropriate DNA vector. The activity of such ribozymes can be augmented by their release from the primary transcript by a second ribozyme (Int. Pat. Appl. Publ. No. WO 93/23569, and Int. Pat. Appl. Publ. No. WO 94/02595, both hereby incorporated by reference; Ohkawa *et al.*, 1992; Taira *et al.*, 1991; and Ventura *et al.*, 1993).

Ribozymes may be added directly, or can be complexed with cationic lipids, lipid complexes, packaged within liposomes, or otherwise delivered to target cells. The RNA or RNA complexes can be locally administered to relevant tissues *ex vivo*, or *in vivo* through injection, aerosol inhalation, infusion pump or stent, with or without their incorporation in biopolymers.

Ribozymes may be designed as described in Int. Pat. Appl. Publ. No. WO 93/23569 and Int. Pat. Appl. Publ. No. WO 94/02595, each specifically incorporated herein by reference) and synthesized to be tested *in vitro* and *in vivo*, as deseribed. Such ribozymes can also be optimized for delivery. While specific examples are provided, those in the art will recognize that equivalent RNA targets in other species can be utilized when necessary.

Hammerhead or hairpin ribozymes may be individually analyzed by computer folding (Jaeger *et al.*, 1989) to assess whether the ribozyme sequences fold

into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. Varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 or so bases on each arm are able to bind to, or otherwise interaet with, the target RNA.

Ribozymes of the hammerhead or hairpin motif may be designed to anneal to various sites in the mRNA message, and can be chemically synthesized. The method of synthesis used follows the procedure for normal RNA synthesis as deseribed in Usman *et al.* (1987) and in Scaringe *et al.* (1990) and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5′-end, and phosphoramidites at the 3′-end. Average stepwise coupling yields are typically >98%. Hairpin ribozymes may be synthesized in two parts and annealed to reconstruct an active ribozyme (Chowrira and Burke, 1992). Ribozymes may be modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2′-amino, 2′-C-allyl, 2′-flouro, 2′-o-methyl, 2′-H (for a review see *e.g.,* Usman and Cedergren, 1992). Ribozymes may be purified by gel electrophoresis using general methods or by high pressure liquid chromatography and resuspended in water.

Ribozyme activity can be optimized by altering the length of the ribozyme binding arms, or chemically synthesizing ribozymes with modifications that prevent their degradation by serum ribonucleases (see *e.g.*, Int. Pat. Appl. Publ. No. WO 92/07065; Perrault *et al*, 1990; Pieken *et al.*, 1991; Usman and Cedergren, 1992; Int. Pat. Appl. Publ. No. WO 93/15187; Int. Pat. Appl. Publ. No. WO 91/03162; Eur. Pat. Appl. Publ. No. 92110298.4; U. S. Patent 5,334,711; and Int. Pat. Appl. Publ. No. WO 94/13688, which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules), modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times and reduce chemical requirements.

Sullivan *et al.* (Int. Pat. Appl. Publ. No. WO 94/02595) describes the general methods for delivery of enzymatic RNA molecules. Ribozymes may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable

nanocapsules, and bioadhesive microspheres. For some indications, ribozymes may be directly delivered *ex vivo* to cells or tissues with or without the aforementioned vehicles. Alternatively, the RNA/vehicle combination may be locally delivered by direct inhalation, by direct injection or by use of a catheter, infusion pump or stent. Other routes of delivery include, but are not limited to, intravascular, intramuscular, subcutaneous or joint injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of ribozyme delivery and administration are provided in Int. Pat. Appl. Publ. No. WO 94/02595 and Int. Pat. Appl. Publ. No. WO 93/23569, each specifically incorporated herein by reference.

Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA expression vector. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhaneers, silencers, *etc.*) present nearby. Prokaryotic RNA polymerase promoters may also be used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990; Gao and Huang, 1993; Lieber *et al.*, 1993; Zhou *et al.*, 1990). Ribozymes expressed from such promoters can function in mammalian cells (*e.g.* Kashani-Saber *et al.*, 1992; Ojwang *et al.*, 1992; Chen *et al.*, 1992; Yu *et al.*, 1993; L'Huillier *et al.*, 1992; Lisziewicz *et al.*, 1993). Such transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated vectors), or viral RNA vectors (such as retroviral, semliki forest virus, sindbis virus vectors).

Ribozymes may be used as diagnostic tools to examine genetic drift and mutations within diseased cells. They can also be used to assess levels of the target RNA molecule. The close relationship between ribozyme activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By using

multiple ribozymes, one may map nucleotide changes which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These studies will lead to better treatment of the disease progression by affording the possibility of combinational therapies (*e.g.*, multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other *in vitro* uses of ribozymes are well known in the art, and include detection of the presence of mRNA associated with an IL-5 related condition. Such RNA is detected by determining the presenee of a eleavage product after treatment with a ribozyme using standard methodology.

PEPTIDE NUCLEIC ACIDS

In certain embodiments, the inventors contemplate the use of peptide nueleie acids (PNAs) in the practice of the methods of the invention. PNA is a DNA mimic in which the nucleobases are attached to a pseudopeptide backbone (Good and Nielsen, 1997). PNA is able to be utilized in a number methods that traditionally have used RNA or DNA. Often PNA sequences perform better in techniques than the corresponding RNA or DNA sequences and have utilities that are not inherent to RNA or DNA. A review of PNA including methods of making, characteristics of, and methods of using, is provided by Corey (1997) and is incorporated herein by reference. As such, in certain embodiments, one may prepare PNA sequences that are complementary to one or more portions of the ACE mRNA sequence, and sueh PNA compositions may be used to regulate, alter, decrease, or reduce the translation of ACE-specific mRNA, and thereby alter the level of ACE activity in a host cell to which such PNA compositions have been administered.

PNAs have 2-aminoethyl-glyeine linkages replacing the normal phosphodiester backbone of DNA (Nielsen *et al.*, 1991; Hanvey *et al.*, 1992; Hyrup and Nielsen, 1996; Neilsen, 1996). This chemistry has three important consequences: firstly, in contrast to DNA or phosphorothioate oligonucleotides, PNAs are neutral

molecules; secondly, PNAs are achiral, which avoids the need to develop a stereoselective synthesis; and thirdly, PNA synthesis uses standard Boc (Dueholm *et al.*, 1994) or Fmoc (Thomson *et al.*, 1995) protocols for solid-phase peptide synthesis, although other methods, including a modified Merrifield method, have been used (Christensen *et al.*, 1995).

PNA monomers or ready-made oligomers are commercially available from PerSeptive Biosystems (Framingham, MA). PNA syntheses by either Boc or Fmoe protocols are straightforward using manual or automated protocols (Norton *et al.*, 1995). The manual protocol lends itself to the production of chemically modified PNAs or the simultaneous synthesis of families of closely related PNAs.

As with peptide synthesis, the success of a particular PNA synthesis will depend on the properties of the chosen sequence. For example, while in theory PNAs can incorporate any combination of nucleotide bases, the presence of adjacent purines can lead to deletions of one or more residues in the product. In expectation of this difficulty, it is suggested that, in producing PNAs with adjaeent purines, one should repeat the coupling of residues likely to be added inefficiently. This should be followed by the purification of PNAs by reverse-phase high-pressure liquid chromatography (Norton *et al.*, 1995) providing yields and purity of product similar to those observed during the synthesis of peptides.

Modifications of PNAs for a given application may be accomplished by coupling amino acids during solid-phase synthesis or by attaching compounds that contain a carboxylic acid group to the exposed N-terminal amine. Alternatively, PNAs can be modified after synthesis by coupling to an introduced lysine or cysteine. The ease with which PNAs can be modified facilitates optimization for better solubility or for specific functional requirements. Once synthesizcd, the identity of PNAs and their derivatives can be confirmed by mass spectrometry. Several studies have made and utilized modifications of PNAs (Norton *et al.*, 1995; Haaima *et al.*, 1996; Stetsenko *et al.*, 1996; Petersen *et al.*, 1995; Ulmann *et al.*, 1996; Koch *et al.*, 1995; Orum *et al.*, 1995; Footer *et al.*, 1996; Griffith *et al.*, 1995; Kremsky *et al.*, 1996; Pardridge *et al.*, 1995; Boffa *et al.*, 1995; Landsdorp *et al.*, 1996; Gambacorti-Passerini *et al.*, 1996; Armitage *et al.*, 1997; Seeger *et al.*, 1997; Ruskowski *et al.*, 1997). U.S. Patent No. 5,700,922 discusses PNA-DNA-PNA chimeric molecules and their uses in diagnostics,

modulating protein in organisms, and treatment of conditions susceptible to therapeutics.

In contrast to DNA and RNA, which contain negatively charged linkages, the PNA backbone is neutral. In spite of this dramatic alteration, PNAs recognize complementary DNA and RNA by Watson-Crick pairing (Egholm *et al.*, 1993), validating the initial modeling by Nielsen *et al.* (1991). PNAs lack 3′ to 5′ polarity and can bind in either parallel or antiparallel fashion, with the antiparallel mode being preferred (Egholm *et al.*, 1993).

Hybridization of DNA oligonucleotides to DNA and RNA is destabilized by electrostatic repulsion between the negatively charged phosphate backbones of the complementary strands. By contrast, the absence of charge repulsion in PNA-DNA or PNA-RNA duplexes increases the melting temperature (T_m) and reduces the dependence of T_m on the concentration of mono- or divalent cations (Nielsen *et al.*, 1991). The enhanced rate and affinity of hybridization are significant because they are responsible for the surprising ability of PNAs to perform strand invasion of complementary sequences within relaxed double-stranded DNA. In addition, the efficient hybridization at inverted repeats suggests that PNAs can recognize secondary structure effectively within double-stranded DNA. Enhanced recognition also occurs with PNAs immobilized on surfaces, and Wang *et al.* have shown that support-bound PNAs can be used to detect hybridization events (Wang *et al.*, 1996).

One might expect that tight binding of PNAs to complementary sequences would also increase binding to similar (but not identical) sequences, reducing the sequence specificity of PNA recognition. As with DNA hybridization, however, selective recognition can be achieved by balancing oligomer length and incubation temperature. Moreover, selective hybridization of PNAs is encouraged by PNA-DNA hybridization being less tolerant of base mismatches than DNA-DNA hybridization. For example, a single mismatch within a 16 bp PNA-DNA duplex can reduce the T_m by up to 15°C (Egholm *et al.*, 1993). This high level of discrimination has allowed the development of several PNA-based strategies for the analysis of point mutations (Wang *et al.*, 1996; Carlsson *et al.*, 1996; Thiede *et al.*, 1996; Webb and Hurskainen, 1996; Perry-O'Keefe *et al.*, 1996).

High-affinity binding provides clear advantages for moleeular recognition and the development of new applications for PNAs. For example, 11-13 nucleotide PNAs inhibit the activity of telomerase, a ribonucleo-protein that extends telomere ends using an essential RNA template, while the analogous DNA oligomers do not (Norton *et al.*, 1996).

Neutral PNAs are more hydrophobic than analogous DNA oligomers, and this can lead to difficulty solubilizing them at neutral pH, especially if the PNAs have a high purine content or if they have the potential to form secondary structures. Their solubility can be enhanced by attaching one or more positive charges to the PNA termini (Nielsen *et al.*, 1991).

Findings by Allfrey and colleagues suggest that strand invasion will occur spontaneously at sequences within chromosomal DNA (Boffa *et al.*, 1995; Boffa *et al.*, 1996). These studies targeted PNAs to triplet repeats of the nucleotides CAG and used this recognition to purify transcriptionally active DNA (Boffa *et al.*, 1995) and to inhibit transcription (Boffa *et al.*, 1996). This result suggests that if PNAs can be delivered within cells then they will have the potential to be general sequence-specifie regulators of gene expression. Studies and reviews concerning the use of PNAs as antisense and anti-gene agents include Nielsen *et al.* (1993b), Hanvey *et al.* (1992), and Good and Nielsen (1997). Koppelhus *et al.* (1997) have used PNAs to inhibit HIV-1 inverse transcription, showing that PNAs may be used for antiviral therapies.

Methods of charaeterizing the antisense binding properties of PNAs are discussed in Rose (1993) and Jensen *et al.* (1997). Rose uses capillary gel electrophoresis to determine binding of PNAs to their complementary oligonucleotide, measuring the relative binding kinetics and stoichiometry. Similar types of measurements were made by Jensen *et al.* using BIAcore™ technology.

Other applications of PNAs include use in DNA strand invasion (Nielsen *et al.*, 1991), antisense inhibition (Hanvey *et al.*, 1992), mutational analysis (Orum *et al.*, 1993), enhancers of transcription (Mollegaard *et al.*, 1994), nucleic acid purifieation (Orum *et al.*, 1995), isolation of transcriptionally active genes (Boffa *et al.*, 1995), blocking of transcription factor binding (Vickers *et al.*, 1995), genome cleavage (Veselkov *et al.*, 1996), biosensors (Wang *et al.*, 1996), *in situ* hybridization (Thisted *et al.*, 1996), and in a alternative to Southern blotting (Perry-O'Keefe, 1996).

POLYPEPTIDE COMPOSITIONS AND USES

The present invention, in other aspects, provides polypeptide compositions. Generally, a polypeptide of the invention will be an isolated polypeptide (or an epitope, variant, or active fragment thereof) derived from a mammalian species. Preferably, the polypeptide is encoded by a polynucleotide sequence disclosed herein or a sequence which hybridizes under moderately stringent conditions to a polynucleotide sequence disclosed herein. Alternatively, the polypeptide may be defined as a polypeptide which comprises a contiguous amino acid sequence from an amino acid sequence disclosed herein, or which polypeptide comprises an entire amino acid sequence disclosed herein.

Likewise, a polypeptide composition of the present invention is understood to comprise one or more polypeptides that are capable of eliciting antibodies that are immunologically reactive with one or more polypeptides encoded by one or more contiguous nucleic acid sequences contained in SEQ ID NO:1-48, 114-121, and 125-138, or to active fragments, or to variants thereof, or to one or more nucleic acid sequences which hybridize to one or more of these sequences under conditions of moderate to high stringency.

As used herein, an active fragment of a polypeptide includes a whole or a portion of a polypeptide which is modified by conventional techniques, *e.g.*, mutagenesis, or by addition, deletion, or substitution, but which active fragment exhibits substantially the same structure function, antigenicity, etc., as a polypeptide as described herein.

In certain illustrative embodiments, the polypeptides of the invention will comprise at least an immunogenic portion of a Chlamydia protein or a variant thereof, as described herein. Proteins that are Chlamydia proteins generally also react detectably within an immunoassay (such as an ELISA) with antisera from a patient with a Chlamydial infection. Polypeptides as described herein may be of any length. Additional sequences derived from the native protein and/or heterologous sequences may be present, and such sequences may (but need not) possess further immunogenic or antigenic properties.

An "immunogenic portion," as used herein is a portion of a protein that

is recognized (*i.e.,* specifically bound) by a B-cell and/or T-cell surface antigen receptor. Such immunogenic portions generally comprise at least 5 amino acid residues, more preferably at least 10, and still more preferably at least 20 amino acid residues of a Chlamydia protein or a variant thereof. Certain preferred immunogenic portions include peptides in which an N-terminal leader sequence and/or transmembrane domain have been deleted. Other preferred immunogenic portions may contain a small N- and/or C-terminal deletion (*e.g.,* 1-30 amino acids, preferably 5-15 amino acids), relative to the mature protein.

Immunogenic portions may generally be identified using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3rd ed., 243-247 (Raven Press, 1993) and references cited therein. Such techniques include screening polypeptides for the ability to react with antigen-specific antibodies, antisera and/or T-cell lines or clones. As used herein, antisera and antibodies are "antigen-specific" if they specifically bind to an antigen (*i.e.,* they react with the protein in an ELISA or other immunoassay, and do not reaet detectably with unrelated proteins). Such antisera and antibodies may be prepared as described herein, and using well known techniques. An immunogenic portion of a native Chlamydia protein is a portion that reacts with such antisera and/or T-cells at a level that is not substantially less than the reactivity of the full length polypeptide (*e.g.,* in an ELISA and/or T-cell reaetivity assay). Such immunogenic portions may react within such assays at a level that is similar to or greater than the reactivity of the full length polypeptide. Such screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow and Lane, *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory, 1988. For example, a polypeptide may be immobilized on a solid support and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected using, for example, ^{125}I-labeled Protein A.

As noted above, a composition may comprise a variant of a native Chlamydia protein. A polypeptide "variant," as used herein, is a polypeptide that differs from a native Chlamydia protein in one or more substitutions, deletions, additions and/or insertions, such that the immunogenicity of the polypeptide is not substantially diminished. In other words, the ability of a variant to react with antigen-specific

antisera may be enhanced or unchanged, relative to the native protein, or may be diminished by less than 50%, and preferably less than 20%, relative to the native protein. Such variants may generally be identified by modifying one of the above polypeptide sequences and evaluating the reactivity of the modified polypeptide with antigen-specific antibodies or antisera as described herein. Preferred variants include those in which one or more portions, such as an N-terminal leader sequence or transmembrane domain, have been removed. Other preferred variants include variants in which a small portion (*e.g.*, 1-30 amino acids, preferably 5-15 amino acids) has been removed from the N- and/or C-terminal of the mature protein.

Polypeptide variants encompassed by the present invention include those exhibiting at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or more identity (determined as described above) to the polypeptides disclosed herein.

Preferably, a variant contains conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartie acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes. In a preferred embodiment, variant polypeptides differ from a native sequence by substitution, deletion or addition of five amino acids or fewer. Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenicity, secondary structure and hydropathic nature of the polypeptide.

As noted above, polypeptides may comprise a signal (or leader) sequence at the N-terminal end of the protein, which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (*e.g.,* poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

Polypeptides may be prepared using any of a variety of well known techniques. Recombinant polypeptides eneoded by DNA sequences as deseribed above may be readily prepared from the DNA sequences using any of a variety of expression vectors known to those of ordinary skill in the art. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast, and higher eukaryotic cells, such as mammalian cells and plant cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line such as COS or CHO. Supernatants from suitable host/vector systems which secrete recombinant protein or polypeptide into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant polypeptide.

Portions and other variants having less than about 100 amino acids, and generally less than about 50 amino acids, may also be generated by synthetic means, using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. *See* Merrifield, *J. Am. Chem. Soc. 85*:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems Division (Foster City, CA), and may be operated according to the manufacturer's instructions.

Within certain specific embodiments, a polypeptide may be a fusion protein that comprises multiple polypeptides as described herein, or that eomprises at

least one polypeptide as described herein and an unrelated sequence, such as a known Chlamydia protein. A fusion partner may, for example, assist in providing T helper epitopes (an immunological fusion partner), preferably T helper epitopes recognized by humans, or may assist in expressing the protein (an expression enhancer) at higher yields than the native recombinant protein. Certain preferred fusion partners are both immunological and expression enhancing fusion partners. Other fusion partners may be selected so as to increase the solubility of the protein or to enable the protein to be targeted to desired intracellular compartments. Still further fusion partners include affinity tags, which facilitate purification of the protein.

Fusion proteins may generally be prepared using standard techniques, including chemical conjugation. Preferably, a fusion protein is expressed as a recombinant protein, allowing the production of inereased levels, relative to a non-fused protein, in an expression system. Briefly, DNA sequences encoding the polypeptide components may be assembled separately, and ligated into an appropriate expression vector. The 3' end of the DNA sequence encoding one polypeptide component is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide component so that the reading frames of the sequences are in phase. This permits translation into a single fusion protein that retains the biological activity of both component polypeptides.

A peptide linker sequence may be employed to separate the first and second polypeptide components by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion protein using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al., *Gene 40*:39-46, 1985; Murphy et al., *Proc. Natl. Acad. Sci. USA 83*:8258-8262, 1986; U.S. Patent No. 4,935,233 and U.S.

Patent No. 4,751,180. The linker sequence may generally be from 1 to about 50 amino acids in length. Linker sequences are not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference.

The ligated DNA sequenees are operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements responsible for expression of DNA are located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons required to end translation and transcription termination signals are only present 3' to the DNA sequence encoding the second polypeptide.

Fusion proteins are also provided. Such proteins comprise a polypeptide as described herein together with an unrelated immunogenic protein. Preferably the immunogenic protein is capable of eliciting a recall response. Examples of such proteins include tetanus, tuberculosis and hepatitis proteins (*see*, for example, Stoute et al. *New Engl. J. Med.*, *336*:86-91, 1997).

Within preferred embodiments, an immunological fusion partner is derived from protein D, a surface protein of the gram-negative bacterium Haemophilus influenza B (WO 91/18926). Preferably, a protein D derivative comprises approximately the first third of the protein (*e.g.,* the first N-terminal 100-110 amino acids), and a protein D derivative may be lipidated. Within certain preferred embodiments, the first 109 residues of a Lipoprotein D fusion partner is included on the N-terminus to provide the polypeptide with additional exogenous T-cell epitopes and to increase the expression level in *E. coli* (thus functioning as an expression enhaneer). The lipid tail ensures optimal presentation of the antigen to antigen presenting eells. Other fusion partners include the non-structural protein from influenzae virus, NS1 (hemaglutinin). Typically, the N-terminal 81 amino acids are used, although different fragments that include T-helper epitopes may be used.

In another embodiment, the immunological fusion partner is the protein known as LYTA, or a portion thereof (preferably a C-terminal portion). LYTA is derived from *Streptococcus pneumoniae*, which synthesizes an N-acetyl-L-alanine amidase known as amidase LYTA (encoded by the LytA gene; *Gene 43*:265-292, 1986). LYTA is an autolysin that specifically degrades certain bonds in the peptidoglycan

backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E. coli* C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LYTA fragment at the amino terminus has been deseribed (*see Biotechnology 10*:795-798, 1992). Within a preferred embodiment, a repeat portion of LYTA may be incorporated into a fusion protein. A repeat portion is found in the C-terminal region starting at residue 178. A particularly preferred repeat portion incorporates residues 188-305.

In general, polypeptides (including fusion proteins) and polynucleotides as described herein are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its original environment. For example, a naturally-occurring protein is isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, such polypeptides are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of the natural environment.

ILLUSTRATIVE THERAPEUTIC COMPOSITIONS AND USES

In another aspect, the present invention provides methods for using one or more of the above polypeptides or fusion proteins (or polynucleotides encoding such polypeptides or fusion proteins) to induce proteetive immunity against Chlamydial infection in a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may be afflicted with a disease, or may be free of detectable disease and/or infection. In other words, protective immunity may be induced to prevent or treat Chlamydial infection.

In this aspect, the polypeptide, fusion protein or polynucleotide molecule is generally present within a pharmaceutical composition or a vaccine. Pharmaceutical compositions may comprise one or more polypeptides, each of which may contain one or more of the above sequences (or variants thereof), and a physiologically acceptable carrier. Vaccines may comprise one or more of the above polypeptides and an immunostimulant, such as an adjuvant or a liposome (into which the polypeptide is incorporated). Such pharmaceutical compositions and vaccines may also contain other

Chlamydia antigens, either incorporated into a combination polypeptide or present within a separate polypeptide.

Alternatively, a vaccine may contain polynucleotides encoding one or more polypeptides or fusion proteins as described above, such that the polypeptide is generated *in situ*. In such vaccines, the polynucleotides may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacterial and viral expression systems. Appropriate nucleic acid expression systems contain the necessary polynucleotide sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as *Bacillus-Calmette-Guerrin*) that expresses an immunogenic portion of the polypeptide on its cell surface. In a preferred embodiment, the polynucleotides may be introduced using a viral expression system (*e.g.*, vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective) virus. Techniques for incorporating polynucleotides into such expression systems are well known to those of ordinary skill in the art. The polynucleotides may also be administered as "naked" plasmid vectors as described, for example, in Ulmer et al., *Science 259*:1745-1749, 1993 and reviewed by Cohen, *Science 259*:1691-1692, 1993.Techniques for incorporating DNA into such vectors are well known to those of ordinary skill in the art. A retroviral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a targeting moiety, such as a gene that encodes a ligand for a receptor on a specific target cell, to render the vector target specific. Targeting may also be accomplished using an antibody, by methods known to those of ordinary skill in the art.

Other formulations for therapeutic purposes include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*i.e.*, an artificial membrane vesicle). The uptake of naked polynucleotides may be increased by incorporating the polynucleotides into and/or onto biodegradable beads, which are efficiently transported into the cells. The preparation and use of such systems is well known in the art.

In a related aspect, a polynucleotide vaccine as described above may be administered simultaneously with or sequentially to either a polypeptide of the present invention or a known *Chlamydia* antigen. For example, administration of polynucleotides encoding a polypeptide of the present invention, either "naked" or in a delivery system as described above, may be followed by administration of an antigen in order to enhance the protective immune effect of the vaccine.

Polypeptides and polynucleotides disclosed herein may also be employed in adoptive immunotherapy for the treatment of *Chlamydial* infection. Adoptive immunotherapy may be broadly classified into either active or passive immunotherapy. In active immunotherapy, treatment relies on the *in vivo* stimulation of the endogenous host immune system with the administration of immune response-modifying agents (for example, vaccines, baeterial adjuvants, and/or cytokines).

In passive immunotherapy, treatment involves the delivery of biologic reagents with established immune reactivity (such as effector cells or antibodies) that can directly or indirectly mediate anti-*Chlamydia* effects and does not neeessarily depend on an intact host immune system. Examples of effector cells include T lymphocytes (for example, CD8+ cytotoxic T-lymphocyte, CD4+ T-helper), killer cells (such as Natural Killer cells, lymphokine-activated killer cells), B cells, or antigen presenting cells (such as dendritic cells and macrophages) expressing the disclosed antigens. The polypeptides disclosed herein may also be used to generate antibodies or anti-idiotypic antibodies (as in U.S. Patent No. 4,918,164), for passive immunotherapy.

The predominant method of procuring adequate numbers of T-cells for adoptive immunotherapy is to grow immune T-cells *in vitro*. Culture conditions for expanding single antigen-specifie T-eells to several billion in number with retention of antigen recognition *in vivo* are well known in the art. These *in vitro* eulture conditions typically utilize intermittent stimulation with antigen, often in the presence of cytokines, such as IL-2, and non-dividing feeder cells. As noted above, the immunoreactive polypeptides described herein may be used to rapidly expand antigen-specific T cell cultures in order to generate sufficient number of cells for immunotherapy. In particular, antigen-presenting cells, such as dendritic, macrophage, monocyte, fibroblast, or B-cells, may be pulsed with immunoreactive polypeptides, or polynucleotide sequence(s) may be introduced into antigen presenting cells, using a

variety of standard techniques well known in the art. For example, antigen presenting cells may be transfected or transduced with a polynucleotide sequence, wherein said sequence contains a promoter region appropriate for increasing expression, and can be expressed as part of a recombinant virus or other expression system. Several viral vectors may be used to transduce an antigen presenting cell, including pox virus, vaccinia virus, and adenovirus; also, antigen presenting cells may be transfected with polynucleotide sequences disclosed herein by a variety of means, including gene-gun technology, lipid-mediated delivery, electroporation, osmotic shock, and particlate delivery mechanisms, resulting in efficient and acceptable expression levels as determined by one of ordinary skill in the art. For cultured T-cells to be effective in therapy, the cultured T-cells must be able to grow and distribute widely and to survive long term *in vivo*. Studies have demonstrated that cultured T-cells can be induced to grow *in vivo* and to survive long term in substantial numbers by repeated stimulation with antigen supplemented with IL-2 (see, for example, Cheever, M., *et al*, "Therapy With Cultured T Cells: Principles Revisited," *Immunological Reviews, 157*:177, 1997).

The polypeptides disclosed herein may also be employed to generate and/or isolate chlamydial-reactive T-cells, which can then be administered to the patient. In one technique, antigen-specific T-cell lines may be generated by *in vivo* immunization with short peptides corresponding to immunogenic portions of the disclosed polypeptides. The resulting antigen specific CD8+ or CD4+ T-cell clones may be isolated from the patient, expanded using standard tissue culture techniques, and returned to the patient.

Alternatively, peptides corresponding to immunogenic portions of the polypeptides may be employed to generate *Chlamydia* reactive T cell subsets by selective *in vitro* stimulation and expansion of autologous T cells to provide antigen-specific T cells which may be subsequently transferred to the patient as described, for example, by Chang *et al, (Crit. Rev. Oncol. Hematol., 22*(3), 213, 1996). Cells of the immune system, such as T cells, may be isolated from the peripheral blood of a patient, using a commercially available cell separation system, such as Isolex™ System, available from Nexell Therapeutics, Inc. Irvine, CA. The separated cells are stimulated with one or more of the immunoreactive polypeptides contained within a delivery vehicle, such as a microsphere, to provide antigen-specific T cells. The population of

antigen-specifie T cells is then expanded using standard techniques and the cells are administered back to the patient.

In other embodiments, T-cell and/or antibody receptors specific for the polypeptides disclosed herein can be cloned, expanded, and transferred into other vectors or effector cells for use in adoptive immunotherapy. In particular, T cells may be transfected with the appropriate genes to express the variable domains from chlamydia speeific monoclonal antibodies as the extracellular reeognition elements and joined to the T cell receptor signaling chains, resulting in T cell activation, specific lysis, and cytokine release. This enables the T cell to redirect its specificity in an MHC-independent manner. See for example, Eshhar, Z., *Cancer Immunol Immunother*, 45(3-4):131-6, 1997 and Hwu, P., et al, *Cancer Res*, 55(15):3369-73, 1995. Another embodiment may include the transfection of chlamydia antigen specific alpha and beta T cell receptor ehains into alternate T cells, as in Cole, DJ, et al, *Cancer Res*, 55(4):748-52, 1995.

In a further embodiment, syngeneic or autologous dendritic cells may be pulsed with peptides eorresponding to at least an immunogenic portion of a polypeptide disclosed herein. The resulting antigen-specific dendritic cells may either be transferred into a patient, or employed to stimulate T cells to provide antigen-specific T cells which may, in turn, be administered to a patient. The use of peptide-pulsed dendritic cells to generate antigen-specific T cells and the subsequent use of such antigen-specific T cells to eradicate disease in a murine model has been demonstrated by Cheever et al, *Immunological Reviews, 157*:177, 1997). Additionally, vectors expressing the disclosed polynucleotides may be introduced into stem cells taken from the patient and clonally propagated *in vitro* for autologous transplant back into the same patient.

Within certain aspects, polypeptides, polynucleotides, T cells and/or binding agents disclosed herein may be incorporated into pharmaceutical compositions or immunogenic compositions (*i.e.,* vaccines). Alternatively, a pharmaceutical composition may comprise an antigen-presenting cell (*e.g.* a dendritie cell) transfected with a *Chlamydial* polynucleotide such that the antigen presenting cell expresses a *Chlamydial* polypeptide. Pharmaceutical compositions comprise one or more such compounds and a physiologically acceptable carrier. Vaccines may comprise one or more such compounds and an immunostimulant. An immunostimulant may be any

substance that enhances or potentiates an immune response to an exogenous antigen. Examples of immunostimulants include adjuvants, biodegradable microspheres (*e.g.,* polylactic galactide) and liposomes (into which the compound is incorporated; *see e.g.,* Fullerton, U.S. Patent No. 4,235,877). Vaccine preparation is generally described in, for example, M.F. Powell and M.J. Newman, eds., "Vaccine Design (the subunit and adjuvant approach)," Plenum Press (NY, 1995). Pharmaceutical compositions and vaccines within the scope of the present invention may also contain other compounds, which may be biologically active or inactive. For example, one or more immunogenic portions of other *Chlamydial* antigens may be present, either incorporated into a fusion polypeptide or as a separate compound, within the composition or vaccine.

A pharmaceutical composition or vaccine may contain DNA encoding one or more of the polypeptides as described above, such that the polypeptide is generated *in situ.* As noted above, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. Numerous gene delivery techniques are well known in the art, such as those described by Rolland, *Crit. Rev. Therap. Drug Carrier Systems 15*:143-198, 1998, and references cited therein. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as *Bacillus-Calmette-Guerrin*) that expresses an immunogenic portion of the polypeptide on its cell surface or secretes such an epitope.

In a preferred embodiment, the DNA may be introduced using a viral expression system (*e.g.,* vaccinia or other pox virus, retrovirus, adenovirus, baculovirus, togavirus, bacteriophage, and the like), which often involves the use of a non-pathogenic (defective), replication competent virus.

For example, many viral expression vectors are derived from viruses of the retroviridae family. This family includes the murine leukemia viruses, the mouse mammary tumor viruses, the human foamy viruses, Rous sarcoma virus, and the immunodeficiency viruses, including human, simian, and feline. Considerations when designing retroviral expression vectors are discussed in Comstock *et al.* (1997).

Excellent murine leukemia virus (MLV)-based viral expression vectors have been developed by Kim *et al.* (1998). In creating the MLV vectors, Kim *et al.* found that the entire *gag* sequence, together with the immediate upstream region, could be deleted without significantly affecting viral packaging or gene expression. Further, it was found that nearly the entire U3 region could be replaced with the immediately-early promoter of human cytomegalovirus without deleterious effects. Additionally, MCR and internal ribosome entry sites (IRES) could be added without adverse effects. Based on their observations, Kim *et al.* have designed a series of MLV-based expression vectors comprising one or more of the features described above.

As more has been learned about human foamy virus (HFV), characteristics of HFV that are favorable for its use as an expression vector have been discovered. These characteristics include the expression of pol by splicing and start of translation at a defined initiation codon. Other aspects of HFV viral expression vectors are reviewed in Bodem *et al.* (1997).

Murakami *et al.* (1997) describe a Rous sarcoma virus (RSV)-based replication-competent avian retrovirus vectors, IR1 and IR2 to express a heterologous gene at a high level. In these vectors, the IRES derived from encephalomyocarditis virus (EMCV) was inserted between the *env* gene and the heterologous gene. The IR1 vector retains the splice-acceptor site that is present downstream of the *env* gene while the IR2 veetor lacks it. Murakami *et al.* have shown high level expression of several different heterologous genes by these vectors.

Recently, a number of lentivirus-based retroviral expression vectors have been developed. Kafri *et al.* (1997) have shown sustained expression of genes delivered directly into liver and muscle by a human immunodeficiency virus (HIV)-based expression vector. One benefit of the system is the inherent ability of HIV to transduce non-dividing cells. Because the viruses of Kafri *et al.* are pseudotyped with vesicular stomatitis virus G glycoprotein (VSVG), they can transduce a broad range of tissues and cell types.

A large number of adenovirus-based expression vectors have been developed, primarily due to the advantages offered by these vectors in gene therapy applications. Adenovirus expression vectors and methods of using such vectors are the subject of a number of United States patents, including United States Patent No.

5,698,202, United States Patent No. 5,616,326, United States Patent No. 5,585,362, and United States Patent No. 5,518,913, all incorporated herein by reference.

Additional adenoviral constructs are described in Khatri *et al.* (1997) and Tomanin *et al.* (1997). Khatri *et al.* describe novel ovine adenovirus expression vectors and their ability to infect bovine nasal turbinate and rabbit kidney cells as well as a range of human cell type, including lung and foreskin fibroblasts as well as liver, prostate, breast, colon and retinal lines. Tomanin *et al.* deseribe adenoviral expression vectors containing the T7 RNA polymerase gene. When introduced into cells containing a heterologous gene operably linked to a T7 promoter, the vectors were able to drive gene expression from the T7 promoter. The authors suggest that this system may be useful for the cloning and expression of genes encoding cytotoxie proteins.

Poxviruses are widely used for the expression of heterologous genes in mammalian cells. Over the years, the vectors have been improved to allow high expression of the heterologous gene and simplify the integration of multiple heterologous genes into a single molecule. In an effort to diminish cytopathic effects and to increase safety, vaceinia virus mutant and other poxviruses that undergo abortive infeetion in mammalian cells are receiving special attention (Oertli *et al.*, 1997). The use of poxviruses as expression vectors is reviewed in Carroll and Moss (1997).

Togaviral expression vectors, which includes alphaviral expression vectors have been used to study the strueture and funetion of proteins and for protein produetion purposes. Attraetive features of togaviral expression veetors are rapid and efficient gene expression, wide host range, and RNA genomes (Huang, 1996). Also, recombinant vaccines based on alphaviral expression vectors have been shown to induce a strong humoral and cellular immune response with good immunologieal memory and proteetive effects (Tubulekas *et al.*, 1997). Alphaviral expression veetors and their use are discussed, for example, in Lundstrom (1997).

In one study, Li and Garoff (1996) used Semliki Forest virus (SFV) expression vectors to express retroviral genes and to produee retroviral particles in BHK-21 cells. The particles produced by this method had protease and reverse transcriptase activity and were infectious. Furthermore, no helper virus could be detected in the virus stocks. Therefore, this system has features that are attractive for its use in gene therapy protocols.

Baculoviral expression vectors have traditionally been used to express heterologous proteins in inseet cells. Examples of proteins include mammalian chemokine receptors (Wang *et al.,* 1997), reporter proteins such as green fluorescent protein (Wu *et al.,* 1997), and FLAG fusion proteins (Wu *et al.,* 1997; Koh *et al.,* 1997). Recent advances in baculoviral expression vector technology, including their use in virion display vectors and expression in mammalian cells is reviewed by Possee (1997). Other reviews on baculoviral expression vectors include Jones and Morikawa (1996) and O'Reilly (1997).

Other suitable viral expression systems are disclosed, for example, in Fisher-Hoeh et al., *Proc. Natl. Acad. Sci. USA 86*:317-321, 1989; Flexner et al., *Ann. N.Y. Acad. Sci. 569*:86-103, 1989; Flexner et al., *Vaccine 8*:17-21, 1990; U.S. Patent Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Patent No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, *Biotechniques 6*:616-627, 1988; Rosenfeld et al., *Science 252*:431-434, 1991; Kolls et al., *Proc. Natl. Acad. Sci. USA 91*:215-219, 1994; Kass-Eisler et al., *Proc. Natl. Acad. Sci. USA 90*:11498-11502, 1993; Guzman et al., *Circulation 88*:2838-2848, 1993; and Guzman et al., *Cir. Res. 73*:1202-1207, 1993. Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. In other systems, the DNA may be introduced as "naked" DNA, as described, for example, in Ulmer et al., *Science 259*:1745-1749, 1993 and reviewed by Cohen, *Science 259*:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the eells.

It will be apparent that a vaceine may comprise a polynucleotide and/or a polypeptide component, as desired. It will also be apparent that a vaccine may contain pharmaceutically acceptable salts of the polynucleotides and/or polypeptides provided herein. Such salts may be prepared from pharmaceutically acceptable non-toxic bases, including organic bases (*e.g.,* salts of primary, secondary and tertiary amines and basic amino acids) and inorganic bases (*e.g.,* sodium, potassium, lithium, ammonium, calcium and magnesium salts). While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. Compositions of the present invention may be formulated for any appropriate manner of administration,

including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous or intramuscular administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (*e.g.,* polylactate polyglycolate) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

Such compositions may also comprise buffers (*e.g.,* neutral buffered saline or phosphate buffered saline), carbohydrates (*e.g.,* glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, bacteriostats, chelating agents such as EDTA or glutathione, adjuvants (*e.g.,* aluminum hydroxide), solutes that render the formulation isotonic, hypotonic or weakly hypertonic with the blood of a recipient, suspending agents, thickening agents and/or preservatives. Alternatively, compositions of the present invention may be formulated as a lyophilizate. Compounds may also be encapsulated within liposomes using well known technology.

Any of a variety of immunostimulants may be employed in the vaccines of this invention. For example, an adjuvant may be included. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, *Bortadella pertussis* or *Mycobacterium tuberculosis* derived proteins. Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ); AS-2 (SmithKline Beecham, Philadelphia, PA); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable mierospheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF or interleukin-2, -7, or -12, may also be used as adjuvants.

Within the vaccines provided herein, under select circumstances, the

adjuvant composition may be designed to induce an immune response predominantly of the Th1 type or Th2 type. High levels of Th1-type cytokines (*e.g.*, IFN-γ, TNFα, IL-2 and IL-12) tend to favor the induction of cell mediated immune responses to an administered antigen. In contrast, high levels of Th2-type cytokines (*e.g.*, IL-4, IL-5, IL-6 and IL-10) tend to favor the induction of humoral immune responses. Following application of a vaccine as provided herein, a patient will support an immune response that includes Th1- and Th2-type responses. Within a preferred embodiment, in which a response is predominantly Th1-type, the level of Th1-type cytokines will increase to a greater extent than the level of Th2-type cytokines. The levels of these cytokines may be readily assessed using standard assays. For a review of the families of cytokines, see Mosmann and Coffman, *Ann. Rev. Immunol.* 7:145-173, 1989.

Preferred adjuvants for use in eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A (3D-MPL), together with an aluminum salt. MPL adjuvants are available from Corixa Corporation (Seattle, WA; *see* US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Sueh oligonucleotides are well known and are deseribed, for example, in WO 96/02555 and WO 99/33488. Immunostimulatory DNA sequences are also described, for example, by Sato et al., *Science 273*:352, 1996. Another preferred adjuvant is a saponin, preferably QS21 (Aquila Biopharmaceuticals Inc., Framingham, MA), which may be used alone or in combination with other adjuvants. For example, an enhanced system involves the combination of a monophosphoryl lipid A and saponin derivative, such as the combination of QS21 and 3D-MPL as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other preferred formulations eomprise an oil-in-water emulsion and tocopherol. A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil-in-water emulsion is described in WO 95/17210.

Other preferred adjuvants include Montanide ISA 720 (Seppic, France), SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (*e.g.*, SBAS-2 or SBAS-4, available from SmithKline Beecham, Rixensart, Belgium), Detox (Corixa Corporation; Seattle, WA), RC-529 (Corixa

Corporation; Seattle, WA) and other aminoalkyl glucosaminide 4-phosphates (AGPs), such as those described in pending U.S. Patent Application Serial Nos. 08/853,826 and 09/074,720, the disclosures of which are incorporated herein by reference in their entireties.

Any vaccine provided herein may be prepared using well known methods that result in a combination of antigen, immunostimulant and a suitable carrier or excipient. The eompositions described herein may be administered as part of a sustained release formulation (*i.e.,* a formulation such as a capsule, sponge or gel (composed of polysaccharides, for example) that effects a slow release of compound following administration). Such formulations may generally be prepared using well known technology (*see, e.g.*, Coombes et al., *Vaccine 14*:1429-1438, 1996) and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a polypeptide, polynucleotide or antibody dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane.

Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. Such carriers include microparticles of poly(lactide-co-glycolide), as well as polyacrylate, latex, starch, cellulose and dextran. Other delayed-release carriers include supramolecular biovectors, which comprise a non-liquid hydrophilic core (*e.g.,* a cross-linked polysaccharide or oligosaccharide) and, optionally, an external layer comprising an amphiphilic compound, such as a phospholipid (*see e.g.,* U.S. Patent No. 5,151,254 and PCT applications WO 94/20078, WO/94/23701 and WO 96/06638). The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Any of a variety of delivery vehicles may be employed within pharmaceutical compositions and vaccines to faeilitate production of an antigen-specifie immune response that targets *Chlamydia*-infectcd eells. Delivery vehicles include antigen presenting cells (APCs), such as dendritic cells, macrophages, B cells, monocytes and other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified to increase the capacity for presenting the antigen,

to improve activation and/or maintenance of the T cell response, to have anti-*Chlamydia* effects *per se* and/or to be immunologically compatible with the receiver (*i.e.,* matched HLA haplotype). APCs may generally be isolated from any of a variety of biological fluids and organs, and may be autologous, allogeneic, syngeneic or xenogeneic cells.

Certain preferred embodiments of the present invention use dendritic cells or progenitors thereof as antigen-presenting cells. Dendritic cells are highly potent APCs (Banchereau and Steinman, *Nature 392*:245-251, 1998) and have been shown to be effective as a physiological adjuvant for eliciting prophylactic or therapeutic immunity (*see* Timmerman and Levy, *Ann. Rev. Med. 50*:507-529, 1999). In general, dendritic cells may be identified based on their typical shape (stellate *in situ*, with marked cytoplasmic processes (dendrites) visible *in vitro*), their ability to take up, proeess and present antigens with high efficiency, and their ability to activate naïve T cell responses. Dendritic cells may, of course, be engineered to express specific cell-surface receptors or ligands that are not commonly found on dendritic cells *in vivo* or *ex vivo*, and such modified dendritic cells are contemplated by the present invention. As an alternative to dendritic cells, secreted vesicles antigen-loaded dendritic cells (called exosomes) may be used within a vaccine (*see* Zitvogel et al., *Nature Med. 4*:594-600, 1998).

Dendritic cells and progenitors may be obtained from peripheral blood, bone marrow, lymph nodes, spleen, skin, umbilical cord blood or any other suitable tissue or fluid. For example, dendritic cells may be differentiated *ex vivo* by adding a combination of cytokines such as GM-CSF, IL-4, IL-13 and/or TNFα to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or bone marrow may be differentiated into dendritic cells by adding to the culture medium combinations of GM-CSF, IL-3, TNFα, CD40 ligand, LPS, flt3 ligand and/or other compound(s) that induce differentiation, maturation and proliferation of dendritic cells.

Dendritic cells are conveniently categorized as "immature" and "mature" cells, which allows a simple way to discriminate between two well characterized phenotypes. However, this nomenclature should not be construed to exclude all possible intermediate stages of differentiation. Immature dendritic cells are

characterized as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fcγ reeeptor and mannose receptor. The mature phenotype is typically characterized by a lower expression of these markers, but a high expression of cell surface molecules responsible for T cell activation such as class I and class II MHC, adhesion molecules (*e.g.,* CD54 and CD11) and costimulatory molecules (*e.g.,* CD40, CD80, CD86 and 4-1BB).

APCs may generally be transfected with a polynucleotide encoding a *Chlamydial* protein (or portion or other variant thereof) such that the *Chlamydial* polypeptide, or an immunogenic portion thereof, is expressed on the cell surface. Such transfection may take place *ex vivo*, and a composition or vaccine comprising such transfected cells may then be used for therapeutie purposes, as described herein. Alternatively, a gene delivery vehicle that targets a dendritic or other antigen presenting cell may be administered to a patient, resulting in transfection that occurs *in vivo*. *In vivo* and *ex vivo* transfection of dendritic cells, for example, may generally be performed using any methods known in the art, such as those described in WO 97/24447, or the gene gun approach described by Mahvi et al., *Immunology and cell Biology 75:*456-460, 1997. Antigen loading of dendritic cells may be achieved by incubating dendritic cells or progenitor eells with the *Chlamydial* polypeptide, DNA (naked or within a plasmid vector) or RNA; or with antigen-expressing recombinant bacterium or viruses (*e.g.,* vaccinia, fowlpox, adenovirus or lentivirus veetors). Prior to loading, the polypeptide may be covalently conjugated to an immunological partner that provides T cell help (*e.g.,* a carrier molecule). Alternatively, a dendritic cell may be pulsed with a non-conjugated immunological partner, separately or in the presence of the polypeptide.

Routes and frequency of administration of pharmaceutical compositions and vaccines, as well as dosage, will vary from individual to individual. In general, the pharmaceutical compositions and vaccines may be administered by injection (*e.g.,* intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (*e.g.,* by aspiration) or orally. Between 1 and 3 doses may be administered for a 1-36 week period. Preferably, 3 doses are administered, at intervals of 3-4 months, and booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of polypeptide or DNA that, when administered as described above, is capable of raising an immune

response in an immunized patient sufficient to protect the patient from *Chlamydial* infection for at least 1-2 years. In general, the amount of polypeptide present in a dose (or produced *in situ* by the DNA in a dose) ranges from about 1 pg to about 100 mg per kg of host, typically from about 10 pg to about 1 mg, and preferably from about 100 pg to about 1 μg. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (*e.g.*, polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

In general, an appropriate dosage and treatment regimen provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Such a response can be monitored by establishing an improved clinical outcome in treated patients as compared to non-treated patients. Increases in preexisting immune responses to a *Chlamydial* protein generally correlate with an improved clinical outcome. Such immune responses may generally be evaluated using standard proliferation, cytotoxicity or cytokine assays, which may be performed using samples obtained from a patient before and after treatment.

DETECTION AND DIAGNOSIS

In another aspect, the present invention provides methods for using the polypeptides described above to diagnose Chlamydial infection. In this aspect, methods are provided for detecting Chlamydial infection in a biological sample, using one or more of the above polypeptides, either alone or in combination. For clarity, the term "polypeptide" will be used when describing specific embodiments of the inventive

diagnostic methods. However, it will be clear to one of skill in the art that the fusion proteins of the present invention may also be employed in such methods.

As used herein, a "biological sample" is any antibody-containing sample obtained from a patient. Preferably, the sample is whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid or urine. More preferably, the sample is a blood, serum or plasma sample obtained from a patient. The polypeptides are used in an assay, as described below, to determine the presence or absence of antibodies to the polypeptide(s) in the sample, relative to a predetermined cut-off value. The presence of such antibodies indicates previous sensitization to *Chlamydia* antigens which may be indicative of *Chlamydia*-infection.

In embodiments in which more than one polypeptide is employed, the polypeptides used are preferably complementary (*i.e.*, one component polypeptide will tend to detect infection in samples where the infection would not be detected by another component polypeptide). Complementary polypeptides may generally be identified by using each polypeptide individually to evaluate serum samples obtained from a series of patients known to be infected with *Chlamydia*. After determining which samples test positive (as described below) with each polypeptide, combinations of two or more polypeptides may be formulated that are capable of detecting infection in most, or all, of the samples tested.

A variety of assay formats are known to those of ordinary skill in the art for using one or more polypeptides to detect antibodies in a sample. *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988, whieh is incorporated herein by reference. In a preferred embodiment, the assay involves the use of polypeptide immobilized on a solid support to bind to and remove the antibody from the sample. The bound antibody may then be detected using a detection reagent that contains a reporter group. Suitable deteetion reagents include antibodies that bind to the antibody/polypeptide complex and free polypeptide labeled with a reporter group (*e.g.*, in a semi-competitive assay). Alternatively, a competitive assay may be utilized, in which an antibody that binds to the polypeptide is labeled with a reporter group and allowed to bind to the immobilized antigen after incubation of the antigen with the sample. The extent to which components of the sample inhibit the

binding of the labeled antibody to the polypeptide is indicative of the reactivity of the sample with the immobilized polypeptide.

The solid support may be any solid material known to those of ordinary skill in the art to which the antigen may be attached. For example, the solid support may be a test well in a microtiter plate, or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetie particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681.

The polypeptides may be bound to the solid support using a variety of techniques known to those of ordinary skill in the art. In the context of the present invention, the term "bound" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the antigen and functional groups on the support or may be a linkage by way of a cross-linking agent). Binding by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the polypeptide, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of polypeptide ranging from about 10 ng to about 1 μg, and preferably about 100 ng, is sufficient to bind an adequate amount of antigen.

Covalent attachment of polypeptide to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the polypeptide. For example, the polypeptide may be bound to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the polypeptide (*see*, *e.g.*, Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

In certain embodiments, the assay is an enzyme linked immunosorbent assay (ELISA). This assay may be performed by first contacting a polypeptide antigen that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that antibodies to the polypeptide within the sample are allowed

to bind to the immobilized polypeptide. Unbound sample is then removed from the immobilized polypeptide and a detection reagent capable of binding to the immobilized antibody-polypeptide complex is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific detection reagent.

More specifically, once the polypeptide is immobilized on the support as described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin (BSA) or Tween 20™ (Sigma Chemical Co., St. Louis, MO) may be employed. The immobilized polypeptide is then incubated with the sample, and antibody is allowed to bind to the antigen. The sample may be diluted with a suitable dilutent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time (*i.e.*, incubation time) is that period of time that is sufficient to detect the presence of antibody within an HGE-infected sample. Preferably, the contact time is sufficient to achieve a level of binding that is at least 95% of that achieved at equilibrium between bound and unbound antibody. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20™. Detection reagent may then be added to the solid support. An appropriate detection reagent is any compound that binds to the immobilized antibody-polypeptide complex and that can be detected by any of a variety of means known to those in the art. Preferably, the detection reagent contains a binding agent (such as, for example, Protein A, Protein G, immunoglobulin, lectin or free antigen) conjugated to a reporter group. Preferred reporter groups include enzymes (such as horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. The conjugation of binding agent to reporter group may be achieved using standard methods known to those of ordinary skill in the art. Common binding agents may also be purchased conjugated to a variety of reporter groups from many commercial sources (*e.g.*, Zymed Laboratories, San Francisco, CA, and Pierce, Rockford, IL).

The detection reagent is then incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound antibody. An appropriate amount of time may generally be determined from the manufacturer's instructions or by assaying the level of binding that occurs over a period of time. Unbound detection reagent is then removed and bound detection reagent is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of anti-*Chlamydia* antibodies in the sample, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. In one preferred embodiment, the cut-off value is the average mean signal obtained when the immobilized antigen is incubated with samples from an uninfected patient. In general, a sample generating a signal that is three standard deviations above the predetermined cut-off value is considered positive for *Chlamydia*-infection. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., *Clinical Epidemiology: A Basic Science for Clinical Medicine*, Little Brown and Co., 1985, pp. 106-107. Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (*i.e.*, sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (*i.e.*, the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In

general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for Chlamydial infection.

In a related embodiment, the assay is performed in a rapid flow-through or strip test format, wherein the antigen is immobilized on a membrane, such as nitrocellulose. In the flow-through test, antibodies within the sample bind to the immobilized polypeptide as the sample passes through the membrane. A detection reagent (*e.g.*, protein A-colloidal gold) then binds to the antibody-polypeptide complex as the solution containing the detection reagent flows through the membrane. The detection of bound detection reagent may then be performed as described above. In the strip test format, one end of the membrane to which polypeptide is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing detection reagent and to the area of immobilized polypeptide. Concentration of detection reagent at the polypeptide indicates the presence of anti-*Chlamydia* antibodies in the sample. Typically, the concentration of detection reagent at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result. In general, the amount of polypeptide immobilized on the membrane is selected to generate a visually discernible pattern when the biological sample contains a level of antibodies that would be sufficient to generate a positive signal in an ELISA, as discussed above. Preferably, the amount of polypeptide immobilized on the membrane ranges from about 25 ng to about 1 μg, and more preferably from about 50 ng to about 500 ng. Such tests can typically be performed with a very small amount (*e.g.*, one drop) of patient serum or blood.

Of course, numerous other assay protocols exist that are suitable for use with the polypeptides of the present invention. The above descriptions are intended to be exemplary only. One example of an alternative assay protocol which may be usefully employed in such methods is a Western blot, wherein the proteins present in a biological sample are separated on a gel, prior to exposure to a binding agent. Such techniques are well known to those of skill in the art.

BINDING AGENTS AND THEIR USES

The present invention further provides agents, such as antibodies and antigen-binding fragments thereof, that specifically bind to a *Chlamydial* protein. As

used herein, an antibody, or antigen-binding fragment thereof, is said to "specifically bind" to a *Chlamydial* protein if it reacts at a detectable level (within, for example, an ELISA) with a *Chlamydial* protein, and does not react detectably with unrelated proteins under similar conditions. As used herein, "binding" refers to a noncovalent association between two separate molecules such that a complex is formed. The ability to bind may be evaluated by, for example, determining a binding constant for the formation of the complex. The binding constant is the value obtained when the concentration of the complex is divided by the product of the component concentrations. In general, two compounds are said to "bind," in the context of the present invention, when the binding constant for complex formation exceeds about 10^3 L/mol. The binding constant may be determined using methods well known in the art.

Binding agents may be further capable of differentiating between patients with and without a *Chlamydial* infection using the representative assays provided herein. In other words, antibodies or other binding agents that bind to a *Chlamydial* protein will generate a signal indicating the presence of a *Chlamydial* infeetion in at least about 20% of patients with the disease, and will generate a negative signal indicating the absence of the disease in at least about 90% of individuals without infection. To determine whether a binding agent satisfies this requirement, biological samples (*e.g.,* blood, sera, sputum urine and/or tissue biopsies) from patients with and without *Chlamydial* infection (as determined using standard clinical tests) may be assayed as described herein for the presence of polypeptides that bind to the binding agent. It will be apparent that a statistically significant number of samples with and without the disease should be assayed. Each binding agent should satisfy the above eriteria; however, those of ordinary skill in the art will recognize that binding agents may be used in combination to improve sensitivity.

Any agent that satisfies the above requirements may be a binding agent. For example, a binding agent may be a ribosome, with or without a peptide component, an RNA molecule or a polypeptide. In a preferred embodiment, a binding agent is an antibody or an antigen-binding fragment thereof. Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. *See, e.g.,* Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In general, antibodies can be produced by cell culture techniques, including the generation

of monoclonal antibodies as described herein, or via transfection of antibody genes into suitable bacterial or mammalian cell hosts, in order to allow for the production of recombinant antibodies. In one technique, an immunogen comprising the polypeptide is initially injected into any of a wide variety of mammals (*e.g.*, mice, rats, rabbits, sheep or goats). In this step, the polypeptides of this invention may serve as the immunogen without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for an antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol. 6*:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and their culture supernatants tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable

vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in, for example, an affinity chromatography step.

Within certain embodiments, the use of antigen-binding fragments of antibodies may be preferred. Such fragments include Fab fragments, which may be prepared using standard techniques. Briefly, immunoglobulins may be purified from rabbit serum by affinity chromatography on Protein A bead columns (Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988) and digested by papain to yield Fab and Fc fragments. The Fab and Fc fragments may be separated by affinity chromatography on protein A bead columns.

Monoclonal antibodies of the present invention may be coupled to one or more therapeutic agents. Suitable agents in this regard include radionuclides, differentiation inducers, drugs, toxins, and derivatives thereof. Preferred radionuclides include ^{90}Y, ^{123}I, ^{125}I, ^{131}I, ^{186}Re, ^{188}Re, ^{211}At, and ^{212}Bi. Preferred drugs include methotrexate, and pyrimidine and purine analogs. Preferred differentiation inducers include phorbol esters and butyric acid. Preferred toxins include ricin, abrin, diptheria toxin, cholera toxin, gelonin, Pseudomonas exotoxin, Shigella toxin, and pokeweed antiviral protein.

A therapeutic agent may be coupled (*e.g.*, covalently bonded) to a suitable monoclonal antibody either directly or indirectly (*e.g.*, via a linker group). A direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (*e.g.*, a halide) on the other.

Alternatively, it may be desirable to couple a therapeutic agent and an antibody via a linker group. A linker group can function as a spacer to distance an antibody from an agent in order to avoid interference with binding capabilities. A linker group can also serve to increase the chemical reactivity of a substituent on an agent or an antibody, and thus increase the coupling efficiency. An increase in chemical

reactivity may also facilitate the use of agents, or functional groups on agents, which otherwise would not be possible.

It will be evident to those skilled in the art that a variety of bifunctional or polyfunctional reagents, both homo- and hetero-functional (such as those described in the catalog of the Pierce Chemical Co., Rockford, IL), may be employed as the linker group. Coupling may be effected, for example, through amino groups, carboxyl groups, sulfhydryl groups or oxidized carbohydrate residues. There are numerous references deseribing such methodology, *e.g.*, U.S. Patent No. 4,671,958, to Rodwell et al.

Where a therapeutic agent is more potent when free from the antibody portion of the immunoconjugates of the present invention, it may be desirable to use a linker group which is cleavable during or upon internalization into a cell. A number of different cleavable linker groups have been described. The mechanisms for the intracellular release of an agent from these linker groups include cleavage by reduction of a disulfide bond (*e.g.*, U.S. Patent No. 4,489,710, to Spitler), by irradiation of a photolabile bond (*e.g.*, U.S. Patent No. 4,625,014, to Senter et al.), by hydrolysis of derivatized amino acid side chains (*e.g.*, U.S. Patent No. 4,638,045, to Kohn et al.), by serum complement-mediated hydrolysis (*e.g.*, U.S. Patent No. 4,671,958, to Rodwell et al.), and aeid-catalyzed hydrolysis (*e.g.*, U.S. Patent No. 4,569,789, to Blattler et al.).

It may be desirable to couple more than one agent to an antibody. In one embodiment, multiple molecules of an agent are coupled to one antibody molecule. In another embodiment, more than one type of agent may be coupled to one antibody. Regardless of the particular embodiment, immunoconjugates with more than one agent may be prepared in a variety of ways. For example, more than one agent may be coupled directly to an antibody molecule, or linkers whieh provide multiple sites for attachment can be used. Alternatively, a carrier can be used.

A carrier may bear the agents in a variety of ways, ineluding covalent bonding either directly or via a linker group. Suitable carriers include proteins such as albumins (*e.g.*, U.S. Patent No. 4,507,234, to Kato et al.), peptides and polysaccharides such as aminodextran (*e.g.*, U.S. Patent No. 4,699,784, to Shih et al.). A carrier may also bear an agent by noncovalent bonding or by encapsulation, such as within a liposome vesicle (*e.g.*, U.S. Patent Nos. 4,429,008 and 4,873,088). Carriers specific for radionuclide agents include radiohalogenated small molecules and chelating

compounds. For example, U.S. Patent No. 4,735,792 discloses representative radiohalogenated small molecules and their synthesis. A radionuclide chelate may be formed from chelating compounds that include those containing nitrogen and sulfur atoms as the donor atoms for binding the metal, or metal oxide, radionuclide. For example, U.S. Patent No. 4,673,562, to Davison et al. discloses representative chelating compounds and their synthesis.

A variety of routes of administration for the antibodies and immunoconjugates may be used. Typically, administration will be intravenous, intramuscular, subcutaneous or in site-specific regions by appropriate methods. It will be evident that the precise dose of the antibody/immunoconjugate will vary depending upon the antibody used, the antigen density, and the rate of clearance of the antibody.

Antibodies may be used in diagnostic tests to detect the presence of *Chlamydia* antigens using assays similar to those detailed above and other techniques well known to those of skill in the art, thereby providing a method for detecting Chlamydial infection in a patient.

Diagnostic reagents of the present invention may also comprise DNA sequences encoding one or more of the above polypeptides, or one or more portions thereof. For example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify *Chlamydia*-specific cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for a DNA molecule encoding a polypeptide of the present invention. The presence of the amplified cDNA is then detected using techniques well known in the art, such as gel electrophoresis. Similarly, oligonucleotide probes specific for a DNA molecule encoding a polypeptide of the present invention may be used in a hybridization assay to detect the presence of an inventive polypeptide in a biological sample.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1

CD4 T CELL EXPRESSION CLONING FOR THE IDENTIFICATION OF T CELL STIMULATING ANTIGENS FROM CHLAMYDIA TRACHOMATIS SEROVAR E

In this example, a CD4+ T cell expression cloning strategy was used to identify Chlamydia trachomatis antigens recognized by patients enrolled in Corixa Corporation's blood donor program. A genomic library of Chlamydia trachomatis serovar E was constructed and screened with Chlamydia specific T cell lines generated by stimulating PBMCs from these donors. Donor CT1 is a 27 yr. old male whose clinical manifestation was non-gonococcal urethritis and his urine was tested positive for Chlamydia by ligase chain reaction. Donor CT3 is a 43 yr. old male who is asymptomatic and infected with serovar J. Donor CT10 is a 24 yr. old female who is asymptomatic and was exposed to Chlamydia through her partner but did not develop the disease. Donor CT11 is a 24 yr. old female with multiple infections (serovar J, F and E).

Chlamydia specific T-cell lines were generated from donors with chlamydial genital tract infection or donors exposed to chlamydia who did not develop the disease. T cell lines from donor CT-1, CT-3 and CT-10 were generated by stimulating PBMC's with reticulate bodies of C. trachomatis serovar E. T-cell lines from donor CT-11 were generated by stimulating PBMC's with either reticulate bodies or elementary bodies of C. trachomatis serovar E. A randomly sheared genomic library of C. trachomatis serovar E was constructed in lambda Zap II vector and an amplified library plated out in 96 well microtiter plates at a density of 25 clones/well. Bacteria were induced to express the recombinant protein in the presence of 2mM IPTG for 2hr, then pelleted and resuspended in 200ul RPMI/10% FBS. 10 ul of the induced bacterial suspension was transferred to 96 well plates containing autologous monocyte-derived dendritic cells. After a 2 hour incubation, dendritic cells were washed to remove E. coli and the T cells were added. Positive E. coli pools were identified by determining IFN gamma production and proliferation of T cells in the pools. The number of pools identified by each T-cell line is as follows: CT1 line : 30/480 pools; CT3 line : 91/960

pools; CT10 line : 40/480 pools; CT11 line : 51/480 pools. The clones identified using this approach are set forth in SEQ ID NO:1-14.

In another example using substantially the same approach described above, we identified 12 additional T-cell reactive clones from Chlamydia trachomatis serovar E expression screening. Clone E5-E9-3 (CT1 positive) contains a 636 bp insert that encodes partially the ORF for dnaK like gene. Part of this sequence was also identified in clone E1-A5-53. Clone E4-H3-56 (CT1 positive, 463 bp insert) contains a partial ORF for the TSA gene (CT603) on the complementary strand. The insert for clone E2-G12-52 (1265 bp) was identified with the CT11 line. It contains a partial ORF for clpB, a protease ATPase. Another clone identified with the CT11 line, E1-F9-79 (167 bp), contains a partial ORF for the gene CT133 on the complementary strand. CT133 is a predicted rRNA methylase. Clone E4-D2-79 (CT3 positive) contains a 1181 bp insert that is a partial ORF for nrdA gene. The ORF for this gene was also identified in clone E2-B10-52 (CT10 positive). Clone E6-C8-95 contains a 731 bp insert that was identified using the donor lines CT3, CT1, and CT12. This insert has a carboxy terminal half for the gene for the 60 kDa ORF. Clone E7-H11-61 (CT3 positive-1135 bp) has partial inserts for fliA (CT061), tyrS (CT062), TSA (CT603) and a hypothetical protein (CT602). The insert for clone E5-A11-8 (CT10 positive-1736 bp) contains the complete ORF for groES (CT111) and a majority of the ORF for groEL (CT110). Clone E3-F2-37 (CT10, CT3, CT11, and CT12 positive-1377bp insert) contains a partial ORF for gene tRNA-Trp (CT322) and a complete ORF for the gene secE (CT321). E4-G9-75 is another CT10 clone that contains a partial ORF (723 bp insert) for the amino terminal region of the pmpH gene (CT872). Clone E2-D5-89 (516bp) is also a CT10 positive clone that contains a partial ORF for pmpD gene (12). The insert for clone E5-E2-10 (CT10 positive) is 427 bp and contains a partial ORF for the major outer membrane protein omp1.

Example 2

ADDITIONAL CD4 T CELL EXPRESSION CLONING FOR THE IDENTIFICATION OF T CELL STIMULATING ANTIGENS FROM CHLAMYDIA TRACHOMATIS SEROVAR E

Twenty sequences were isolated from single clones using a Chlamydia trachomatis serovar E (Ct E) library expression screening method. Descriptions of how the clones and lines were generated are provided in Example 1.

Clone E5-A8-85 (identified using the CT1 patient line) was found to contain a 1433 bp insert. This insert contains a large region of the C-terminal half of the CT875, a Chlamydia trachomatis hypothetical specific gene that is disclosed in SEQ ID NO:34. Also present in the clone is a partial open reading frame (ORF) of a hypothetical protein CT001 which is on the complementary strand.

The clone E9-G2-93 (identified using the C10 patient line) was shown to contain a 554 bp insert, the sequence of which is disclosed in SEQ ID NO:33. This sequence encodes a partial ORF for CT178, a hypothetical CT protein.

Clone E7-B1-16 (identified using the patient lines CT10, CT3, CT5, CT11, CT13, and CHH037) has a 2577 bp insert, the sequenee of which is disclosed in SEQ ID NO:32. This clone was found to contain three ORFs. The first ORF contains almost the entire ORF for CT694, a Chlamydia trachomatis (CT) specific hypothetical protein. The second ORF is a full length ORF for CT695, another hypothetical CT protein. The third ORF is the N-terminal portion of CT696.

Clone E9-D5-8 (identified using the patient lines CT10, CT1, CT4, and CT11) contains a 393 bp insert, which is disclosed in SEQ ID NO:31. It was found to encode a partial ORF for CT680, the S2 ribosomal protein.

Clone E9-E10-51 (identified using the patient line CT10) contains an 883 bp insert, the sequence of which is disclosed in SEQ ID NO:30. This clone contains two partial ORF. The first of these is for the C-terminal half of CT680, which may show some overlap with the insert present in clone E9-D5-8. The second ORF is the N-terminal partial ORF for CT679, which is the elongation factor TS.

Clone E3-B4-18 (identified using the CT1 patient line) contains a 1224 bp insert, the sequence of which is disclosed in SEQ ID NO:29. This clone contains 4 ORFs. At the N-terminal end of the clone is the complete ORF for CT772, coding for inorganic pyrophosphatase. The second ORF is a small portion of the C-terminal end of CT771, on the complementary frame. The third is a partial ORF of the hypothetical protein, CT191 and the fourth is a partial ORF for CT190, DNA gyrase-B.

Clone E10-B2-57 (identified using the CT10 patient line) contains an 822 bp insert, the sequence of which is disclosed in SEQ ID NO:42. This clone contains the complete ORF for CT066, a hypothetical protein, on the complementary strand.

Clone E3-F3-18 (identified using the CT1 patient line) contains an 1141 bp insert, the sequence of which is disclosed in SEQ ID NO:41. It contains a partial ORF for pmpG (CT871) in frame with the β-gal gene.

Clone E4-D6-21 (identified using the CT3 patient line) contains a 1297 bp insert, the sequence of which is disclosed in SEQ ID NO:40. This clone contains a very small portion of xseA (CT329), the entire ORF for tpiS (CT328) on the complementary strand, and a partial amino terminal ORF for trpC (CT327) on the top frame.

Clone E1-G9-23 (identified using the CT3 patient line) contains an 1180 bp insert, the sequence of which is disclosed in SEQ ID NO:39. This clone contains almost the entire ORF for glycogen synthase (CT798).

Clone E3-A3-31 (identified using the CT1 patient line) contains an 1834 bp insert, the sequence of which is disclosed in SEQ ID NO:38. This clone contains a large region of the hypothetical gene CT622.

Clone E2-F7-11 (identified using both the CT3 and CT10 patient lines) contains a 2093 bp insert, the sequence of which is disclosed in SEQ ID NO:37. This clone contains a large region of the rpoN gene (CT609) in frame with β-gal and the eomplete ORF for the hypothetical gene CT610 on the complementary strand. In addition, it also contains the carboxy-terminal end of CT611, another hypothetical gene.

Clone E7-H11-10 (identified using the CT3 patient line) contains a 1990 bp insert, the sequence of which is disclosed in SEQ ID NO:36. This clone contains the amino terminal partial ORF for CT610, a complete ORF for CT611, another complete ORF for CT612, and a carboxy-terminal portion of CT613. All of these genes are hypothetical and all are present on the complementary strand.

Clone E10-C6-45 (identified using the CT3 patient line) contains a 196 bp insert, the sequence of whieh is disclosed in SEQ ID NO:35. This clone contains a partial ORF for nrdA (CT827) in frame with β-gal. This elone contains a relatively

small insert and has particular utility in determining the epitope of this gene that contributes to the immunogenicity of Serovar E.

Clone E3-H6-10 (identified using the CT12 patient line) contains a 3734 bp insert, the sequence of which is disclosed in SEQ ID NO:48. This clone contains ORFs for a series of hypothetical proteins. It contains the partial ORFs for CT223 and CT229 and the complete ORFs for CT224, CT225, CT226, CT227, and CT228.

Clone E4-C3-40 (identified using the CT10 patient line) contains a 2044 bp insert, the sequence of which is disclosed in SEQ ID NO:47. This clone contains a partial ORF for nrdA (CT827) and the eomplete ORF for nrdB (CT828).

Clone E2-D8-19 (identified using the CT1 patient line) contains a 2010 bp insert, the sequence of which is disclosed in SEQ ID NO:46. This clone contains ORF from the Chlamydia trachomatis plasmid as well as containing partial ORFs for ORF3 and ORF6, and complete ORFs for ORF4 and ORF5.

Clone E3-D10-46 (identified using the patient lines CT1, CT3, CT4, CT11, and CT12) contains a 1666 bp insert, the sequence of which is identified in SEQ ID NO: 45. This clone contains a partial ORF for CT770 (fab F), a complete ORF for CT771 (hydrolase/phosphatase homologue), a complete ORF for CT772 (ppa, inorganic phosphatase), and a partial ORF for CT773 (Idh, Leucine dehydrogenase).

Clone E10-H8-1 (identified using both the CT3 and CT10 patient lines) contains an 1862 bp insert, the sequence of which is disclosed in SEQ ID NO:44. It contains the partial ORFs for CT871 (pmpG) as well as CT872 (pmpH).

Clone E3-F3-7 (identified using the CT1 patient line) contains a 1643 bp insert, the sequence of which is identified in SEQ ID NO:43. It contains the partial ORFs for both CT869 (pmpE) and CT870 (pmpF).

EXAMPLE 3

ADDITIONAL CD4 T CELL EXPRESSION CLONING FOR THE IDENTIFICATION OF T CELL STIMULATING ANTIGENS FROM CHLAMYDIA TRACHOMATIS SEROVAR E

The T cell line CHH037 was generated from a 22 year-old healthy female sero-negative for Chlamydia. This line was used to screen the Chlamydia

trachomatis serovar E library. Nineteen clones were identified from this screen, as described below.

Clone E7-B12-65, contains an 1179 bp insert, the sequence of which is disclosed in SEQ ID NO:114. It contains the complete ORF of the gene for Malate dehydrogenase (CT376) on the complementary strand.

Clone E4-H9-83 contains a 772 bp insert, the sequence of which is identified in SEQ ID NO:115. It contains the partial ORF for the heat shock protein GroEL (CT110).

Clone E9-B10-52 contains a 487 bp insert, the sequence of which is identified in SEQ ID NO:116. It contains a partial ORF for the gene yscC (CT674), a general secretion pathway protein.

Clone E7-A7-79 contains a 1014 bp insert, the sequence of which is disclosed in SEQ ID NO:117. It contains the complete ORF for the histone like development gene, hctA (CT743) and a partial ORF for the rRNA methyltransferase gene ygcA (CT742).

Clone E2-D11-18 contains a 287 bp insert, the sequence of which is disclosed in SEQ ID NO:118. It contains the partial ORF for hctA (CT743).

Clone E9-H6-15, identified using the CT3 line, contains a 713 bp insert the sequence of which is disclosed in SEQ ID NO:125. It contains the partial ORF of the pmpB gene (CT413).

Clone E3-D10-87, identified using the CT1 line, contains a 780 bp insert, the sequence of which is disclosed in SEQ ID NO:126. It contains the partial ORF for CT388, a hypothetical gene, on the complementary strand, and a partial ORF for CT389, another hypothetical protein.

Clone E9-D6-43, identified using the CT3 line, contains a 433 bp insert, the sequence of which is disclosed in SEQ ID NO:127. It contains a partial ORF for CT858.

Clone E3-D10-4, identified using the CT1 line, contains an 803 bp insert, the sequence of which is disclosed in SEQ ID NO:128. It contains a partial ORF for pGP3-D, an ORF encoded on the plasmid pCHL1.

Clone E3-G8-7, identified using the CT1 line, contains an 842 bp insert, the sequence of which is disclosed in SEQ ID NO:129. It contains partial ORFs for CT557 (Lpda) and CT558 (LipA).

Clone E3-F11-32, identified using the CT1 line, contains an 813 bp insert, the sequence of which is disclosed in SEQ ID NO:130. It contains a partial ORF for pmpD (CT812).

Clone E2-F8-5, identified using the CT12 line, contains a 1947 bp insert, the sequence of which is disclosed in SEQ ID NO:131. It contains a complete ORF for the 15 kDa ORF (CT442) and a partial ORF for the 60 kDa ORF (CT443).

Clone E2-G4-39, identified using the CT12 line, contains a 1278 bp insert, the sequence of which is disclosed in SEQ ID NO:132. It contains the partial ORF of the 60kDa ORF (CT443).

Clone E9-D1-16, identified using the CT10 line, contains a 916 bp insert, the sequence of which is disclosed in SEQ ID NO:133. It contains the partial ORF for the pmpH (CT872).

Clone E3-F3-6, identified using the CT1 line, contains a 751 bp insert, the sequence of which is disclosed in SEQ ID NO:134. It contains the partial ORFs, all on he complementary strand, for genes accB (CT123), L13 ribosomal (CT125), and S9 ribosomal (CT126).

Clone E2-D4-70, identified using the CT12 line, contains a 410 bp insert, the sequence of which is disclosed in SEQ ID NO:135. It contains the partial ORF for the pmpC gene (CT414).

Clone E5-A1-79, identified using the CT1 line, contains a 2719 bp insert, the sequence of which is disclosed in SEQ ID NO:136. It contains a partial ORF for ydhO (CT127), a complete ORF for S9 ribosomal gene (CT126 on the complementary strand), a complete ORF for the L13 ribosomal gene (CT125 on the complementary strand) and a partial ORF for accC (CT124 on the complementary strand).

Clone E1-F7-16, identified using the lines CT12, CT3, and CT11, contains a 2354 bp insert, the sequence of which is disclosed in SEQ ID NO:137. It contains a partial ORF of the ftsH gene (CT841) and the entire ORF for the pnp gene (CT842) on the complementary strand.

Clone E1-D8-62, identified using the CT12 line, contains an 898 bp insert, the sequence of which is disclosed in SEQ ID NO:138. It contains partial ORFs for the ftsH gene (CT841) and for the pnp gene (CT842).

EXAMPLE 4

EXPRESSION OF CHLAMYDIA TRACOMATIS RECOMBINANT PROTEINS

Several Chlamydia trachomatis serovar E specific genes were cloned into pET17b. This plasmid incorporates a 6X histidine tag at the N-terminal to allow for expression and purification of recombinant protein.

Two full-length recombinant proteins, CT622 and CT875, were expressed in E. coli. Both of these genes were identified using CtLGVII expression screening, but the serovar E homologues were expressed. The primers used to amplify these genes were based on serovar D sequences. The genes were amplified using serovar E genomic DNA as the template. Once amplified, the fragments were cloned in pET-17b with a N-terminal 6X-His Tag. After transforming the recombinant plasmid in XL-I blue cells, the DNA was prepared and the clones fully sequeneed. The DNA was then transformed into the expression host BL21-pLysS cells (Novagen) for production of the recombinant proteins. The proteins were induced with IPTG and purified on Ni-NTA agarose using standard methods. The DNA sequences for CTE622 and CTE875 are disclosed in SEQ ID NO:28 and 27 respectively, and their amino acid sequences are disclosed in SEQ ID NO: 140 and 139, respectively

Five additional Chlamydia trachomatis genes were cloned. The Chalmydia trachomatis specific protein CT694, the protein CT695, and the L1 ribosomal protein, the DNA sequences of which are disclosed in SEQ ID NO:119, 120 and 121 respectively. The protein sequences of these 6X-histidine recombinant proteins are disclosed in SEQ ID NO: 122 (CT694), 123 (CT695), and 124 (L1 ribosomal protein). The genes CT875 and CT622, from serovar E were also cloned using pET17b as 6X-His fusion proteins. These recombinant proteins were expressed and purified and their the amino acid sequences diselosed in SEQ ID NO:139 and 140, respectively.

EXAMPLE 5

RECOMBINANT CHLAMYDIAL ANTIGENS RECOGNIZED BY T CELL LINES

Patient T cell lines were generated from the following donors: CT1, CT2, CT3, CT4, CT5, CT6, CT7, CT8, CT9, CT10, CT11, CT12, CT13, CT14, CT15, and CT16. A summary of their details is included in Table II.

Table II: C. *trachomatis* patients

Patients	Gender	Age	Clinical Manifestation	Serovar	IgG titer	Multiple Infections
CT1	M	27	NGU	LCR	Negative	No
CT2	M	24	NGU	D	Negative	E
CT3	M	43	Asymptomatic Shed Eb Dx was HPV	J	Ct 1:512 Cp 1:1024 Cps 1:256	No
CT4	F	25	Asymptomatic Shed Eb	J	Ct 1:1024	Y
CT5	F	27	BV	LCR	Ct 1:256 Cp 1:256	F/F
CT6	M	26	Perinial rash Discharge, dysuria	G	Cp 1:1024	N
CT7	F	29	BV Genital ulcer	E	Ct 1:512 Cp 1:1024	N
CT8	F	24	Not Known	LCR	Not tested	NA
CT9	M	24	asymptomatic	LCR	Ct 1:128 Cp 1:128	N

CT10	F	20	Mild itch vulvar	negative	negative	12/1/98
CT11	F	21	BV Abnormal pap smear	J	Ct 1:512	F/F/J/E/E PID 6/96
CT12	M	20	asymptomatic	LCR	Cp 1:512	N
CT13	F	18	BV, gonorrhea, Ct vaginal discharge, dysuria	G	Ct 1:1024	N
CT14	M	24	NGU	LCR	Ct 1:256 Cp 1:256	N
CT15	F	21	Muco-purulint cervicitis Vaginal discharge	culture	Ct 1:256 Ct IgM 1:320 Cp 1:64	N
CT16	M	26	Asymptomatic/ contact	LCR	NA	N
CL8	M	38	No clinical history of disease	negative	negative	No

NGU=Non-Gonococcal Urethritis; BV=Bacterial Vaginosis; CT=Chlamydia trachomatis; Cp=Chlamydia pneumoniae; Eb=Chlamydia elementary bodies; HPV=human papiloma virus; Dx=diagnosis; PID=pelvic inflammatory disease; LCR=Ligase change reaction.

PBMC were collected from a second series of donors and T cell lines have been generated from a sub-set of these. A summary of the details for three such T cell lines is listed in the table below.

Table III: Normal Donors				
Donor	**Gender**	**Age**	**CT IgG Titer**	**CP IgG Titer**
CHH011	F	49	1:64	1:16
CHH037	F	22	0	0
CHH042	F	25	0	1:16

Donor CHH011 is a healthy 49 year old female donor sero-negaitve for *C. trachomatis*. PBMC produced higher quantities of IFN-gamma in response to *C. trachomatis* elementary bodies as compared to *C. pneumoniae* elementary bodies, indicating a *C. trachomatis*-specific response. Donor CHH037 is a 22 year old healthy female donor sero-negative for C. trachomatis. PBMC poruced higher quantities of IFN-gamma in response to *C. trachomatis* elementary bodies as compared to *C. pneumoniae* elementary bodies, indicating a *C. trachomatis*-specific response. CHH042 is a 25 year old healthy female donor with an IgG titer of 1:16 to *C. pneumoniae*. PBMC produced higher quantities of IFN-gamma in response to *C. trachomatis* elementary bodies as compared to *C. pneumoniae* elementary bodies, indicating a *C. trachomatis*-specific response.

Recombinant proteins for several *Chlamydia trachomatis* genes were generated as described above. Sequences for MOMP was derived from serovar F. The genes CT875, CT622, pmp-B-2, pmpA, and CT529 were derived from serovar E and sequences for the genes gro-EL, Swib, pmpD, pmpG, TSA, CT610, pmpC, pmpE, S13, lpdA, pmpI, and pmpH-C were derived from LII.

Several of the patient and donor lines described above were tested against the recombinant Chlamydia proteins. Table IV summarizes the results of the T cell responses to the recombinant Chlamydia proteins.

Table IV: Recombinant Chlamydia Antigens Recognized By T Cell Lines

Antigen	Serovar	#of hits	CL 8 L2	CT 10 E	CT 1 E	CT 3 E	CT 4 L2	CT 5 E	CT 11 E	CT 12 E	CT 13 E	CHH-011 E	CHH-037 E
gro-EL (CT110)	L2	10	-	+	+	+	+	+	+	+	+	+	+
MompF (CT681)	F	10	-	+	+	+	+	+	+	+	+	+	+
CT875	E	8	-	+	+	-	+	+	+	+	+	-	+
SWIB (CT460)	L2	8	+	+	-	+	-	+	-	+	+	+	+
pmpD (CT812)	L2	5	-	+	+	+	+	-	-	+	+	-	-
pmpG (CT871)	L2	6	-	+	+	-	+	+	nt	-	+	+	-
TSA (CT603)	L2	6	-	-	+	+	+	+	-	-	+	-	+
CT622	E	3	-	-	+	-	+	-	-	-	+	-	-
CT610	L2	3	-	+	-	+	-	-	-	+	-	-	-
pmpB-2 (CT413)	E	3	-	-	+	+	+	-	-	-	-	-	-
pmpC (CT414)	L2	4	-	-	-	+	-	+	-	+	-	-	+
pmpE (CT869)	L2	3	-	+	+	-	-	-	-	-	+	-	-
S13 (CT509)	L2	2	+	-	-	-	+	-	-	-	-	-	-
lpdA (CT557)	L2	3	-	-	+	+	-	-	-	-	-	+	-
pmpI (CT874)	L2	2	-	-	+	-	-	-	-	-	-	+	-
pmpH-C (CT872)	L2	1	-	-	-	-	-	-	-	+	-	-	-

pmpA (CT412)	E	0	-	-	-	-	-	-	-	-	-	-	-
CT529	E	0	-	-	-	-	-	-	-	-	-	-	-

Although the present invention has been described in some detail by way of illustration and example for purposes of elarity of understanding, changes and modifications ean be carried out without departing from the scope of the invention which is intended to be limited only by the scope of the appended claims.

Claims

What is Claimed:

1. An isolated polynucleotide comprising a sequence selected from the group consisting of:

(a) sequences provided in SEQ ID NO:1-48, 114-121, and 125-138;

(b) complements of the sequences provided in SEQ ID NO: 1-48, 114-121, and 125-138;

(e) sequences consisting of at least 20 contiguous residues of a sequence provided in SEQ ID NO:1-48, 114-121, 125-138;

(d) sequences that hybridize to a sequence provided in SEQ ID NO:1-48, 114-121, and 125-138, under highly stringent conditions;

(e) sequences having at least 95% identity to a sequence of SEQ ID NO:1-48, 114-121, and 125-138;

(f) sequences having at least 99% identity to a sequence of SEQ ID NO: 1-48, 114-121, and 125-138; and

(g) degenerate variants of a sequence provided in SEQ ID NO: 1-48, 114-121, and 125-138.

2. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) sequences encoded by a polynucleotide of claim 1;

(b) sequences having at least 95% identity to a sequence eneoded by a polynucleotide of claim 1; and

(c) sequences having at least 99% identity to a sequence encoded by a polynucleotide of claim 1.

3. An isolated polypeptide eomprising at least an immunogenic fragment of a polypeptide sequence selected from the group consisting of:

(a) a polypeptide sequence set forth in SEQ ID NO: 122-124 and 139-140,

(b) a polypeptide sequence having at least 95% identity with a sequence set forth in SEQ ID NO: 122-124 and 139-140, and

(c) a polypeptide sequence having at least 99% identity with a sequence set forth in SEQ ID NO: 122-124 and 139-140.

4. An expression vector comprising a polynueleotide of claim 1 operably linked to an expression control sequence.

5. A host cell transformed or transfected with an expression vector according to claim 4.

6. An isolated antibody, or antigen-binding fragment thereof, that specifically binds to a polypeptide of claim 2 or claim 3.

7. A method for detecting the presence of Chlamydia in a patient, comprising the steps of:

(a) obtaining a biological sample from the patient;

(b) contacting the biological sample with a binding agent that binds to a polypeptide of claim 2 or claim 3;

(c) detecting in the sample an amount of polypeptide that binds to the binding agent; and

(d) comparing the amount of polypeptide to a predetermined cut-off value and therefrom determining the presence of Chlamydia in the patient.

8. A fusion protein comprising at least one polypeptide aecording to claim 2 or claim 3.

9. An oligonucleotide that hybridizes to a sequence recited in any one of SEQ ID NO: 1-48, 114-121, and 125-138 under highly stringent conditions.

10. A method for stimulating and/or expanding T cells specific for a Chlamydia protein, comprising contacting T cells with at least one eomponent selected from the group consisting of:

(a) a polypeptide according to claim 2 or claim 3;

(b) a polynucleotide according to claim 1; and

(c) an antigen-presenting cell that expresses a polynucleotide according to claim 1,

under conditions and for a time sufficient to permit the stimulation and/or expansion of T cells.

11. An isolated T cell population, comprising T cells prepared according to the method of claim 10.

12. A composition comprising a first component selected from the group consisting of physiologically acceptable carriers and immunostimulants, and a second component selected from the group consisting of:

(a) a polypeptide according to claim 2 or claim 3;

(b) a polynucleotide according to claim 1;

(c) an antibody according to claim 6;

(d) a fusion protein according to claim 8;

(e) a T cell population according to claim 11; and

(f) an antigen presenting cell that expresses a polypeptide according to claim 2 or claim 3.

13. A method for stimulating an immune response in a patient, comprising administering to the patient a composition selected from the group consisting of:

(a) a composition of claim 12;

(b) a polynucleotide sequence of any one of SEQ ID NO:80-94; and

(c) a polypeptide sequence of any one of SEQ ID NO:95-109.

14. A method for the treatment of Chlamydia infection in a patient,

comprising administering to the patient a composition selected from the group consisting of:

(a) a composition of claim 12;

(b) a polynucleotide sequence of any one of SEQ ID NO:80-94; and

(d) a polypeptide sequence of any one of SEQ ID NO:95-109.

15. A method for determining the presence of Chlamydia in a patient, comprising the steps of:

(a) obtaining a biological sample from the patient;

(b) contacting the biological sample with an oligonucleotide according to claim 9;

(c) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide; and

(d) comparing the amount of polynucleotide that hybridizes to the oligonucleotide to a predetermined cut-off value, and therefore determining the presence of the cancer in the patient.

16. A diagnostic kit comprising at least one oligonucleotide according to claim 9.

17. A diagnostic kit comprising at least one antibody according to claim 6 and a detection reagent, wherein the detection reagent comprises a reporter group.

18. A method for the treatment of Chlamydia in a patient, comprising the steps of:

(a) incubating CD4+ and/or CD8+ T cells isolated from a patient with at least one component selected from the group consisting of:

(i) a polypeptide according to any one of claims 2 or 3;

(ii) a polypeptide sequence of any one of SEQ ID NO: 95-109;

(iii) a polynucleotide according to claim 1;

(iv) a polynucleotide sequence of any one of SEQ ID NO:80-94;

(v) an antigen presenting cell that expresses a polypeptide sequence set forth in any one of claims 2 or 3;

(vi) an antigen presenting cell that expresses a polypeptide sequence of any one of SEQ ID NO:95-109, such that the T cells proliferate; and

(b) administering to the patient an effective amount of the proliferated T cells.

SEQUENCE LISTING

<110> Corixa Corporation
Bhatia, Ajay
Probst, Peter
Stromberg, Erika Jean

<120> COMPOUNDS AND METHODS FOR TREATMENT AND DIAGNOSIS OF CHLAMYDIAL INFECTION

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<210> 7
<211> 861
<212> DNA
<213> Chlamydia trachomatis

<400> 7
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<210> 8
<211> 763
<212> DNA
<213> Chlamydia trachomatis

<400> 8
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atcaaagccg gaggcctggg agacgcgcta tacggactag caaaagcttt agccgctaat 180
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<210> 9
<211> 665
<212> DNA
<213> Chlamydia trachomatis

<400> 9
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attta 665

<210> 10
<211> 843
<212> DNA
<213> Chlamydia trachomatis

<400> 10
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<210> 11
<211> 1474
<212> DNA
<213> Chlamydia trachomatis

<400> 11
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gcataaatgt taggtacgtt tgtgcgcatt gtggcatcgg tagggatgac tccgcgttca 180
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<210> 12
<211> 2017
<212> DNA
<213> Chlamydia trachomatis

<400> 12
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tttctcaaca ggtacacgat ggcctttaaa ttctgttttg atggtttcaa gaacaccttc 180
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<210> 13
<211> 1171
<212> DNA
<213> Chlamydia trachomatis

<400> 13
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caaaaaagaa tttcttttt ggctcttttt t 1171

<210> 14
<211> 877
<212> DNA
<213> Chlamydia trachomatis

<400> 14
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<210> 15
<211> 396
<212> DNA
<213> Chlamydia trachomatis serovar E

<400> 15
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gtctcgagca agttttgatg ccgatacgat tcgtat 396

<210> 16
<211> 516
<212> DNA
<213> Chlamydia trachomatis serovar E

<400> 16
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gttcgggagg agctgttcgt atagaggatc atggtaatgt tcttttagaa gcttttggag 120
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aactgaagat tttagattca ggaactcctg tacaag 516

<210> 17
<211> 723
<212> DNA
<213> Chlamydia trachomatis serovar E

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aca 723

<210> 18
<211> 1377
<212> DNA
<213> Chlamydia trachomatis serovar E

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<211> 1135
<212> DNA
<213> Chlamydia trachomatis serovar E

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<210> 21
<211> 731
<212> DNA
<213> Chlamydia trachomatis serovar E

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<210> 22
<211> 1181
<212> DNA
<213> Chlamydia trachomatis serovar E

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<210> 23
<211> 167
<212> DNA
<213> Chlamydia trachomatis serovar E

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<210> 24
<211> 1265
<212> DNA
<213> Chlamydia trachomatis serovar E

<400> 24
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<210> 25
<211> 463
<212> DNA
<213> Chlamydia trachomatis serovar E

<400> 25
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<210> 26
<211> 636
<212> DNA
<213> Chlamydia trachomatis serovar E

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<210> 27
<211> 1797
<212> DNA
<213> Chlamydia trachomatis serE

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<210> 28
<211> 1983
<212> DNA
<213> Chlamydia trachomatis serE

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<212> DNA
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<210> 30
<211> 883
<212> DNA
<213> Chlamydia trachomatis serE

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<210> 31
<211> 393
<212> DNA
<213> Chlamydia trachomatis serE

<400> 31
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<210> 32
<211> 2577
<212> DNA
<213> Chlamydia trachomatis serE

<400> 32
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<210> 33
<211> 554
<212> DNA
<213> Chlamydia trachomatis serE

<400> 33
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ttttttaaa ttaagtgtac ttccagctct tctcggactc tggctatttt ttactcctaa 180
tattcttaac tatttggatt ctctgttat tttatcagat aaaatttgcg gcgtcttttt 240
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ttccgcggca cgag 554

<210> 34
<211> 1433
<212> DNA
<213> Chlamydia trachomatis serE

<400> 34

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<210> 35
<211> 196
<212> DNA
<213> Chlamydia trachomatis

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<400> 35
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caggggatga gcgtcgacgg gctcatgatg tcaatatagc tagctggatt ccagatcttt 180
tcttcaaacg tttaca                                              196
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<210> 36
<211> 1990
<212> DNA
<213> Chlamydia trachomatis

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<400> 36
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<210> 37
<211> 2093
<212> DNA
<213> Chlamydia trachomatis

<400> 37
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<210> 38
<211> 1834

<212> DNA
<213> Chlamydia trachomatis

<400> 38
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<210> 39
<211> 1180
<212> DNA
<213> Chlamydia trachomatis

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<210> 40
<211> 1297
<212> DNA
<213> Chlamydia trachomatis

<400> 40
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caatacacag aactggaatc attccactat ggatagctgc agcagctttt tcagcaagta 480
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<210> 41
<211> 1141
<212> DNA
<213> Chlamydia trachomatis

<400> 41
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t 1141

<210> 42
<211> 822
<212> DNA

<213> Chlamydia trachomatis

<400> 42
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agcttcaacg taagcattcc aaagctccgt acttacaata ttattgcgga tagagcgaat 180
taattctctt tttagtgatg gaagagttt ttgggggctg aagcgagcca aaagatcttt 240
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<210> 43
<211> 1634
<212> DNA
<213> Chlamydia trachomatis

<400> 43
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tacattagtc acaatatttt cattaaaaat aatagcatgg cggtcggcag aatttttgga 180
gttactggtt ccgtctatat agatagcgcc ccccttatta ttggcgatat tgtttataaa 240
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atgattatga tttatgtaaa gattttgagc atgaatggct ccgcctctc ttatttatc 780
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aatggggtga gcat 1634

<210> 44
<211> 1862
<212> DNA
<213> Chlamydia trachomatis

<400> 44
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cg 1862

<210> 45
<211> 1668
<212> DNA
<213> Chlamydia trachomatis

<400> 45
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actottttgca aatagaagac ctacatattg aaggatatga acaggttttg aaagttactt 1620
gcgagtctgt acagttagtt gctgtaattg ctattcatca gacaaaag              1668

<210> 46
<211> 2010
<212> DNA
<213> Chlamydia trachomatis

<400> 46
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<210> 47
<211> 2044
<212> DNA
<213> Chlamydia trachomatis

<400> 47
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tctc 2044

<210> 48
<211> 3734
<212> DNA
<213> Chlamydia trachomatis

<400> 48
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<211> 252
<212> DNA
<213> Chlamydia pneumoniae

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<213> Chlamydia pneumoniae

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<212> DNA
<213> Chlamydia pneumoniae

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<211> 1041
<212> DNA
<213> Chlamydia pneumoniae

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<211> 3135
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<213> Chlamydia pneumoniae

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<213> Chlamydia pneumoniae

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<212> DNA
<213> Chlamydia pneumoniae

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<211> 1983
<212> DNA
<213> Chlamydia pneumoniae

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<212> DNA
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gcaggtgcgg aagctaagcc taaagaatct aagacccgatt ctgtagagcg atggagcatc 180
ttgcgttctg cagtgaatgc tctcatgagt ctggcagata agctgggtat tgcttctagt 240
aacagctcgt cttctactag cagatctgca gacgtggact caacgacagc gaccgcacct 300
acgcctcctc cacccacgtt tgatgattat aagactcaag cgcaaacagc ttacgatact 360
atcttacctc caacatcact agctgacata caggcctgctt tggtgagcct ccaggatgct 420
gtcactaata taaaggatac agcggctact gatgaggaaa ccgcaatcgc tgcggagtgg 480
gaaactaaga atgccgatgc agttaaagtt ggcgcgcaaa ttacagaatt agcgaaatat 540
gcttcggata accaagcgat tcttgactct ttaggtaaac tgacttcctt cgacctctta 600
caggctgctc ttctccaatc tgtagcaaac aataacaaag cagctgagct tcttaaagag 660
atgcaagata acccagtagt cccagggaaa acgcctgcaa ttgctcaatc tttagttgat 720
cagacagatg ctacagcgac acagatagag aaagatggaa atgcgattag ggatgcatat 780
tttgcaggac agaacgctag tggagctgta gaaaatgcta aatctaataa cagtataagc 840
aacatagatt cagctaaagc agcaatcgct actgctaaga cacaaatagc tgaagctcag 900
aaaaagttcc ccgactctcc aattcttcaa gaagcggaac aaatggtaat acaggctgag 960
aaagtcttta aaaatatcaa acctgcagat ggttctgatg ttccaaatcc aggaactaca 1020
gttggaggct ccaagcaaca aggaagtagt attggtagta ttcgtgtttc catgctgtta 1080
gatgatgctg aaaatgagac cgcttccatt ttgatgtctg ggtttcgtca gatgattcac 1140
atgttcaata cggaaaatcc tgattctcaa gctgccccaac aggagctcgc agcacaagct 1200
agagcagcga aagcccgctgg agatgacagt gctgctgcag cgctggcaga tgctcagaaa 1260
gctttagaag cggctctagg taaagctggg caacaacagg gcatactcaa tgctttagga 1320
cagatcgctt ctgctgctgt tgtgagcgca ggagttcctc ccgctgcagc aagttctata 1380
gggtcatctg taaaacagct ttacaagacc tcaaaatcta caggttctga ttataaaaca 1440
cagatatcag caggttatga tgcttacaaa tccatcaatg atgcctatgg tagggcacga 1500
aatgatgcga ctcgtgatgt gataaacaat gtaagtaccc ccgctctcac acgatccgtt 1560
cctagagcac gaacagaagc tcgaggacca gaaaaaacag atcaagccct cgctagggtg 1620
atttctggca atagcagaac tcttggagat gtctatagtc aagttcggc actacaatct 1680
gtaatgcaga tcatccagtc gaatcctcaa gcgaataatg aggagatcag acaaaagctt 1740
acatcggcag tgacaaagcc tccacagttt ggctatcctt atgtgcaact ttctaatgac 1800
tctacacaga agttcatagc taaattagaa agtttgtttg ctgaaggatc taggacagca 1860
gctgaaataa aagcactttc ctttgaaacg aactccttgt ttattcagca ggtgctggtc 1920
aatatcggct ctctatattc tggttatctc caataa 1956

<210> 64
<211> 264
<212> DNA
<213> Chlamydia pneumoniae

<400> 64
atgagtcaaa aaaataaaaa ctctgctttt atgcatcccg tgaatatttc cacagattta 60
gcagttatag ttggcaaggg acctatgccc agaaccgaaa ttgtaaagaa agtttgggaa 120
tacattaaaa aacacaactg tcaggatcaa aaaaataaac gtaatatcct tcccgatgcg 180
aatcttgcca aagtctttgg ctctagtgat cctatcgaca tgttccaaat gaccaaagcc 240
ctttccaaac atattgtaaa ataa 264

<210> 65
<211> 978
<212> PRT
<213> Chlamydia pneumoniae

<400> 65
Met Pro Leu Ser Phe Lys Ser Ser Ser Phe Cys Leu Leu Ala Cys Leu
1 5 10 15
Cys Ser Ala Ser Cys Ala Phe Ala Glu Thr Arg Leu Gly Gly Asn Phe
20 25 30
Val Pro Pro Ile Thr Asn Gln Gly Glu Glu Ile Leu Leu Thr Ser Asp
35 40 45
Phe Val Cys Ser Asn Phe Leu Gly Ala Ser Phe Ser Ser Ser Phe Ile
50 55 60
Asn Ser Ser Ser Asn Leu Ser Leu Leu Gly Lys Gly Leu Ser Leu Thr
65 70 75 80
Phe Thr Ser Cys Gln Ala Pro Thr Asn Ser Asn Tyr Ala Leu Leu Ser
85 90 95
Ala Ala Glu Thr Leu Thr Phe Lys Asn Phe Ser Ser Ile Asn Phe Thr
100 105 110
Gly Asn Gln Ser Thr Gly Leu Gly Gly Leu Ile Tyr Gly Lys Asp Ile
115 120 125
Val Phe Gln Ser Ile Lys Asp Leu Ile Phe Thr Thr Asn Arg Val Ala
130 135 140
Tyr Ser Pro Ala Ser Val Thr Thr Ser Ala Thr Pro Ala Ile Thr Thr
145 150 155 160
Val Thr Thr Gly Ala Ser Ala Leu Gln Pro Thr Asp Ser Leu Thr Val
165 170 175
Glu Asn Ile Ser Gln Ser Ile Lys Phe Phe Gly Asn Leu Ala Asn Phe
180 185 190
Gly Ser Ala Ile Ser Ser Ser Pro Thr Ala Val Val Lys Phe Ile Asn
195 200 205
Asn Thr Ala Thr Met Ser Phe Ser His Asn Phe Thr Ser Ser Gly Gly
210 215 220
Gly Val Ile Tyr Gly Gly Ser Ser Leu Leu Phe Glu Asn Asn Ser Gly
225 230 235 240
Cys Ile Ile Phe Thr Ala Asn Ser Cys Val Asn Ser Leu Lys Gly Val
245 250 255
Thr Pro Ser Ser Gly Thr Tyr Ala Leu Gly Ser Gly Gly Ala Ile Cys
260 265 270
Ile Pro Thr Gly Thr Phe Glu Leu Lys Asn Asn Gln Gly Lys Cys Thr
275 280 285
Phe Ser Tyr Asn Gly Thr Pro Asn Asp Ala Gly Ala Ile Tyr Ala Glu
290 295 300
Thr Cys Asn Ile Val Gly Asn Gln Gly Ala Leu Leu Leu Asp Ser Asn
305 310 315 320
Thr Ala Ala Arg Asn Gly Gly Ala Ile Cys Ala Lys Val Leu Asn Ile
325 330 335
Gln Gly Arg Gly Pro Ile Glu Phe Ser Arg Asn Arg Ala Glu Lys Gly
340 345 350
Gly Ala Ile Phe Ile Gly Pro Ser Val Gly Asp Pro Ala Lys Gln Thr
355 360 365
Ser Thr Leu Thr Ile Leu Ala Ser Glu Gly Asp Ile Ala Phe Gln Gly
370 375 380
Asn Met Leu Asn Thr Lys Pro Gly Ile Arg Asn Ala Ile Thr Val Glu
385 390 395 400
Ala Gly Gly Glu Ile Val Ser Leu Ser Ala Gln Gly Gly Ser Arg Leu
405 410 415
Val Phe Tyr Asp Pro Ile Thr His Ser Leu Pro Thr Thr Ser Pro Ser
420 425 430
Asn Lys Asp Ile Thr Ile Asn Ala Asn Gly Ala Ser Gly Ser Val Val
435 440 445
Phe Thr Ser Lys Gly Leu Ser Ser Thr Glu Leu Leu Pro Ala Asn
450 455 460
Thr Thr Thr Ile Leu Leu Gly Thr Val Lys Ile Ala Ser Gly Glu Leu

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465                 470                 475                 480
Lys Ile Thr Asp Asn Ala Val Val Asn Val Leu Gly Phe Ala Thr Gln
                485                 490                 495
Gly Ser Gly Gln Leu Thr Leu Gly Ser Gly Gly Thr Leu Gly Leu Ala
            500                 505                 510
Thr Pro Thr Gly Ala Pro Ala Ala Val Asp Phe Thr Ile Gly Lys Leu
        515                 520                 525
Ala Phe Asp Pro Phe Ser Phe Leu Lys Arg Asp Phe Val Ser Ala Ser
    530                 535                 540
Val Asn Ala Gly Thr Lys Asn Val Thr Leu Thr Gly Ala Leu Val Leu
545                 550                 555                 560
Asp Glu His Asp Val Thr Asp Leu Tyr Asp Met Val Ser Leu Gln Ser
                565                 570                 575
Pro Val Ala Ile Pro Ile Ala Val Phe Lys Gly Ala Thr Val Thr Lys
            580                 585                 590
Thr Gly Phe Pro Asp Gly Glu Ile Ala Thr Pro Ser His Tyr Gly Tyr
        595                 600                 605
Gln Gly Lys Trp Ser Tyr Thr Trp Ser Arg Pro Leu Leu Ile Pro Ala
    610                 615                 620
Pro Asp Gly Gly Phe Pro Gly Gly Pro Ser Pro Ser Ala Asn Thr Leu
625                 630                 635                 640
Tyr Ala Val Trp Asn Ser Asp Thr Leu Val Arg Ser Thr Tyr Ile Leu
                645                 650                 655
Asp Pro Glu Arg Tyr Gly Glu Ile Val Ser Asn Ser Leu Trp Ile Ser
            660                 665                 670
Phe Leu Gly Asn Gln Ala Phe Ser Asp Ile Leu Gln Asp Val Leu Leu
        675                 680                 685
Ile Asp His Pro Gly Leu Ser Ile Thr Ala Lys Ala Leu Gly Ala Tyr
    690                 695                 700
Val Glu His Thr Pro Arg Gln Gly His Glu Gly Phe Ser Gly Arg Tyr
705                 710                 715                 720
Gly Gly Tyr Gln Ala Ala Leu Ser Met Asn Tyr Thr Asp His Thr Thr
                725                 730                 735
Leu Gly Leu Ser Phe Gly Gln Leu Tyr Gly Lys Thr Asn Ala Asn Pro
            740                 745                 750
Tyr Asp Ser Arg Cys Ser Glu Gln Met Tyr Leu Leu Ser Phe Phe Gly
        755                 760                 765
Gln Phe Pro Ile Val Thr Gln Lys Ser Glu Ala Leu Ile Ser Trp Lys
    770                 775                 780
Ala Ala Tyr Gly Tyr Ser Lys Asn His Leu Asn Thr Thr Tyr Leu Arg
785                 790                 795                 800
Pro Asp Lys Ala Pro Lys Ser Gln Gly Gln Trp His Asn Asn Ser Tyr
                805                 810                 815
Tyr Val Leu Ile Ser Ala Glu His Pro Phe Leu Asn Trp Cys Leu Leu
            820                 825                 830
Thr Arg Pro Leu Ala Gln Ala Trp Asp Leu Ser Gly Phe Ile Ser Ala
        835                 840                 845
Glu Phe Leu Gly Gly Trp Gln Ser Lys Phe Thr Glu Thr Gly Asp Leu
    850                 855                 860
Gln Arg Ser Phe Ser Arg Gly Lys Gly Tyr Asn Val Ser Leu Pro Ile
865                 870                 875                 880
Gly Cys Ser Ser Gln Trp Phe Thr Pro Phe Lys Lys Ala Pro Ser Thr
                885                 890                 895
Leu Thr Ile Lys Leu Ala Tyr Lys Pro Asp Ile Tyr Arg Val Asn Pro
            900                 905                 910
His Asn Ile Val Thr Val Val Ser Asn Gln Glu Ser Thr Ser Ile Ser
        915                 920                 925
Gly Ala Asn Leu Arg Arg His Gly Leu Phe Val Gln Ile His Asp Val
    930                 935                 940
Val Asp Leu Thr Glu Asp Thr Gln Ala Phe Leu Asn Tyr Thr Phe Asp
945                 950                 955                 960
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Gly Lys Asn Gly Phe Thr Asn His Arg Val Ser Thr Gly Leu Lys Ser
                965                 970                 975
Thr Phe


<210> 66
<211> 266
<212> PRT
<213> Chlamydia pneumoniae

<400> 66
Met His Ser Lys Phe Leu Ser Arg Arg Lys Lys Asn Ser Ser His Lys
                5                  10                  15
Glu Glu Thr Ser Trp Asp Cys Ile Ala Ser Ser Tyr Asn Lys Ile Val
            20                  25                  30
Gln Asp Lys Gly His Tyr Tyr His Arg Glu Thr Ile Leu Pro Gln Leu
        35                  40                  45
Leu Pro Ser Leu Thr Leu Gly Ser Lys Ser Ser Val Leu Asp Ile Gly
    50                  55                  60
Cys Gly Gln Gly Phe Leu Glu Arg Ala Leu Pro Lys Glu Cys Arg Tyr
65                  70                  75                  80
Leu Gly Ile Asp Ile Ser Ser Arg Leu Ile Ala Leu Ala Lys Lys Met
                85                  90                  95
Arg Ser Val Asn Ser His Gln Phe Lys Val Ala Asp Leu Ser Lys Arg
            100                 105                 110
Leu Glu Phe Val Glu Pro Thr Leu Phe Ser His Ala Val Ala Ile Leu
        115                 120                 125
Ser Leu Gln Asn Met Glu Phe Pro Gly Glu Ala Ile Arg Asn Thr Ala
    130                 135                 140
Thr Leu Leu Glu Pro Leu Gly Gln Phe Phe Ile Val Leu Asn His Pro
145                 150                 155                 160
Cys Phe Arg Ile Pro Arg Ala Ser Ser Trp His Tyr Asp Glu Asn Lys
                165                 170                 175
Lys Ala Ile Ser Arg His Ile Asp Arg Tyr Leu Ser Pro Met Lys Ile
            180                 185                 190
Pro Ile Met Ala His Pro Gly Gln Lys Asp Ser Pro Ser Thr Leu Ser
        195                 200                 205
Phe His Phe Pro Leu Ser Tyr Trp Phe Lys Glu Leu Ser Ser His Gly
    210                 215                 220
Phe Leu Val Ser Gly Leu Glu Glu Trp Thr Ser Ser Lys Thr Ser Thr
225                 230                 235                 240
Gly Lys Arg Ala Lys Ala Glu Asn Leu Cys Arg Lys Glu Phe Pro Leu
                245                 250                 255
Phe Leu Met Ile Ser Cys Ile Lys Ile Lys
            260                 265


<210> 67
<211> 83
<212> PRT
<213> Chlamydia pneumoniae

<400> 67
Met Lys Gln Gln His Asn Arg Lys Ala Leu Ser Arg Lys Ile Gly Thr
                5                  10                  15
Val Lys Lys Gln Ala Lys Phe Ala Gly Ser Phe Leu Asp Glu Ile Lys
            20                  25                  30
Lys Ile Glu Trp Val Ser Lys His Asp Leu Lys Lys Tyr Ile Lys Val
        35                  40                  45
Val Leu Ile Ser Ile Phe Gly Phe Gly Phe Ala Ile Tyr Phe Val Asp
    50                  55                  60
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Leu Val Leu Arg Lys Ser Ile Thr Cys Leu Asp Gly Ile Thr Thr Phe
65 70 75 80
Leu Phe Gly

<210> 68
<211> 394
<212> PRT
<213> Chlamydia pneumoniae

<400> 68
Met Ser Lys Glu Thr Phe Gln Arg Asn Lys Pro His Ile Asn Ile Gly
1 5 10 15
Thr Ile Gly His Val Asp His Gly Lys Thr Thr Leu Thr Ala Ala Ile
20 25 30
Thr Arg Ala Leu Ser Gly Asp Gly Leu Ala Ser Phe Arg Asp Tyr Ser
35 40 45
Ser Ile Asp Asn Thr Pro Glu Glu Lys Ala Arg Gly Ile Thr Ile Asn
50 55 60
Ala Ser His Val Glu Tyr Glu Thr Pro Asn Arg His Tyr Ala His Val
65 70 75 80
Asp Cys Pro Gly His Ala Asp Tyr Val Lys Asn Met Ile Thr Gly Ala
85 90 95
Ala Gln Met Asp Gly Ala Ile Leu Val Val Ser Ala Thr Asp Gly Ala
100 105 110
Met Pro Gln Thr Lys Glu His Ile Leu Leu Ala Arg Gln Val Gly Val
115 120 125
Pro Tyr Ile Val Val Phe Leu Asn Lys Val Asp Met Ile Ser Gln Glu
130 135 140
Asp Ala Glu Leu Ile Asp Leu Val Glu Met Glu Leu Ser Glu Leu Leu
145 150 155 160
Glu Glu Lys Gly Tyr Lys Gly Cys Pro Ile Ile Arg Gly Ser Ala Leu
165 170 175
Lys Ala Leu Glu Gly Asp Ala Asn Tyr Ile Glu Lys Val Arg Glu Leu
180 185 190
Met Gln Ala Val Asp Asp Asn Ile Pro Thr Pro Glu Arg Glu Ile Asp
195 200 205
Lys Pro Phe Leu Met Pro Ile Glu Asp Val Phe Ser Ile Ser Gly Arg
210 215 220
Gly Thr Val Val Thr Gly Arg Ile Glu Arg Gly Ile Val Lys Val Ser
225 230 235 240
Asp Lys Val Gln Leu Val Gly Leu Gly Glu Thr Lys Glu Thr Ile Val
245 250 255
Thr Gly Val Glu Met Phe Arg Lys Glu Leu Pro Glu Gly Arg Ala Gly
260 265 270
Glu Asn Val Gly Leu Leu Leu Arg Gly Ile Gly Lys Asn Asp Val Glu
275 280 285
Arg Gly Met Val Val Cys Gln Pro Asn Ser Val Lys Pro His Thr Lys
290 295 300
Phe Lys Ser Ala Val Tyr Val Leu Gln Lys Glu Glu Gly Gly Arg His
305 310 315 320
Lys Pro Phe Phe Ser Gly Tyr Arg Pro Gln Phe Phe Phe Arg Thr Thr
325 330 335
Asp Val Thr Gly Val Val Thr Leu Pro Glu Gly Thr Glu Met Val Met
340 345 350
Pro Gly Asp Asn Val Glu Leu Asp Val Glu Leu Ile Gly Thr Val Ala
355 360 365
Leu Glu Glu Gly Met Arg Phe Ala Ile Arg Glu Gly Gly Arg Thr Ile
370 375 380
Gly Ala Gly Thr Ile Ser Lys Ile Asn Ala
385 390

<210> 69
<211> 476
<212> PRT
<213> Chlamydia pneumoniae

<400> 69
Met Arg Ile Val Gln Val Ala Val Glu Phe Thr Pro Ile Val Lys Val
1 5 10 15
Gly Gly Leu Gly Asp Ala Val Ala Ser Leu Ser Lys Glu Leu Ala Lys
20 25 30
Gln Asn Asp Val Glu Val Leu Leu Pro His Tyr Pro Leu Ile Ser Lys
35 40 45
Phe Ser Ser Ser Gln Val Leu Ser Glu Arg Ser Phe Tyr Tyr Glu Phe
50 55 60
Leu Gly Lys Gln Gln Ala Ser Ala Ile Ser Tyr Ser Tyr Glu Gly Leu
65 70 75 80
Thr Leu Thr Ile Ile Thr Leu Asp Ser Gln Ile Glu Leu Phe Ser Thr
85 90 95
Thr Ser Val Tyr Ser Glu Asn Asn Val Val Arg Phe Ser Ala Phe Ala
100 105 110
Ala Ala Ala Ala Tyr Leu Gln Glu Ala Asp Pro Ala Asp Ile Val
115 120 125
His Leu His Asp Trp His Val Gly Leu Leu Ala Gly Leu Leu Lys Asn
130 135 140
Pro Leu Asn Pro Val His Ser Lys Ile Val Phe Thr Ile His Asn Phe
145 150 155 160
Gly Tyr Arg Gly Tyr Cys Ser Thr Gln Leu Leu Ala Ala Ser Gln Ile
165 170 175
Asp Asp Phe His Leu Ser His Tyr Gln Leu Phe Arg Asp Pro Gln Thr
180 185 190
Ser Val Leu Met Lys Gly Ala Leu Tyr Cys Ser Asp Tyr Ile Thr Thr
195 200 205
Val Ser Leu Thr Tyr Val Gln Glu Ile Ile Asn Asp Tyr Ser Asp Tyr
210 215 220
Glu Leu His Asp Ala Ile Leu Ala Arg Asn Ser Val Phe Ser Gly Ile
225 230 235 240
Ile Asn Gly Ile Asp Glu Asp Val Trp Asn Pro Lys Thr Asp Pro Ala
245 250 255
Leu Ala Val Gln Tyr Asp Ala Ser Leu Leu Ser Glu Pro Asp Val Leu
260 265 270
Phe Thr Lys Lys Glu Glu Asn Arg Ala Val Leu Tyr Glu Lys Leu Gly
275 280 285
Ile Ser Ser Asp Tyr Phe Pro Leu Ile Cys Val Ile Ser Arg Ile Val
290 295 300
Glu Glu Lys Gly Pro Glu Phe Met Lys Glu Ile Ile Leu His Ala Met
305 310 315 320
Glu His Ser Tyr Ala Phe Ile Leu Ile Gly Thr Ser Gln Asn Glu Val
325 330 335
Leu Leu Asn Glu Phe Arg Asn Leu Gln Asp Cys Leu Ala Ser Ser Pro
340 345 350
Asn Ile Arg Leu Ile Leu Asp Phe Asn Asp Pro Leu Ala Arg Leu Thr
355 360 365
Tyr Ala Ala Ala Asp Met Ile Cys Ile Pro Ser His Arg Glu Ala Cys
370 375 380
Gly Leu Thr Gln Leu Ile Ala Met Arg Tyr Gly Thr Val Pro Leu Val
385 390 395 400
Arg Lys Thr Gly Gly Leu Ala Asp Thr Val Ile Pro Gly Val Asn Gly
405 410 415
Phe Thr Phe Phe Asp Thr Asn Asn Phe Asn Glu Phe Arg Ala Met Leu

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            420                 425                 430
Ser Asn Ala Val Thr Thr Tyr Arg Gln Glu Pro Asp Val Trp Leu Asn
        435                 440                 445
Leu Ile Glu Ser Gly Met Leu Arg Ala Ser Gly Leu Asp Ala Met Ala
    450                 455                 460
Lys His Tyr Val Asn Leu Tyr Gln Ser Leu Leu Ser
465                 470                 475


<210> 70
<211> 346
<212> PRT
<213> Chlamydia pneumoniae

<400> 70
Met Glu Ala Asp Ile Leu Asp Gly Lys Leu Lys Arg Val Glu Val Ser
                5                  10                  15
Lys Lys Gly Leu Val Asn Cys Asn Gln Val Asp Val Asn Gln Leu Val
            20                  25                  30
Pro Ile Lys Tyr Lys Trp Ala Trp Glu His Tyr Leu Asn Gly Cys Ala
        35                  40                  45
Asn Asn Trp Leu Pro Thr Glu Val Pro Met Ala Arg Asp Ile Glu Leu
    50                  55                  60
Trp Lys Ser Asp Glu Leu Ser Glu Asp Glu Arg Arg Val Ile Leu Leu
65                  70                  75                  80
Asn Leu Gly Phe Phe Ser Thr Ala Glu Ser Leu Val Gly Asn Asn Ile
                85                  90                  95
Val Leu Ala Ile Phe Lys His Ile Thr Asn Pro Glu Ala Arg Gln Tyr
            100                 105                 110
Leu Leu Arg Gln Ala Phe Glu Glu Ala Val His Thr His Thr Phe Leu
        115                 120                 125
Tyr Ile Cys Glu Ser Leu Gly Leu Asp Glu Gly Glu Val Phe Asn Ala
    130                 135                 140
Tyr Asn Glu Arg Ala Ser Ile Arg Ala Lys Asp Asp Phe Gln Met Thr
145                 150                 155                 160
Leu Thr Val Asp Val Leu Asp Pro Asn Phe Ser Val Gln Ser Ser Glu
                165                 170                 175
Gly Leu Gly Gln Phe Ile Lys Asn Leu Val Gly Tyr Tyr Ile Ile Met
            180                 185                 190
Glu Gly Ile Phe Phe Tyr Ser Gly Phe Val Met Ile Leu Ser Phe His
        195                 200                 205
Arg Gln Asn Lys Met Thr Gly Ile Gly Glu Gln Tyr Gln Tyr Ile Leu
    210                 215                 220
Arg Asp Glu Thr Ile His Leu Asn Phe Gly Ile Asp Leu Ile Asn Gly
225                 230                 235                 240
Ile Lys Glu Glu Asn Pro Glu Val Trp Thr Thr Glu Leu Gln Glu Glu
                245                 250                 255
Ile Val Ala Leu Ile Glu Lys Ala Val Glu Leu Glu Ile Glu Tyr Ala
            260                 265                 270
Lys Asp Cys Leu Pro Arg Gly Ile Leu Gly Leu Arg Ser Met Phe
        275                 280                 285
Ile Asp Tyr Val Arg His Ile Ala Asp Arg Arg Leu Glu Arg Ile Gly
    290                 295                 300
Leu Lys Pro Ile Tyr His Ser Arg Asn Pro Phe Pro Trp Met Ser Glu
305                 310                 315                 320
Thr Met Asp Leu Asn Lys Glu Lys Asn Phe Phe Glu Thr Arg Val Thr
                325                 330                 335
Glu Tyr Gln Thr Ala Gly Asn Leu Ser Trp
            340                 345
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<210> 71
<211> 1044
<212> PRT
<213> Chlamydia pneumoniae

<400> 71
Met Val Glu Val Glu Glu Lys His Tyr Thr Ile Val Lys Arg Asn Gly
1 5 10 15
Met Phe Val Pro Phe Asn Gln Asp Arg Ile Phe Gln Ala Leu Glu Ala
20 25 30
Ala Phe Arg Asp Thr Arg Ser Leu Glu Thr Ser Ser Pro Leu Pro Lys
35 40 45
Asp Leu Glu Glu Ser Ile Ala Gln Ile Thr His Lys Val Val Lys Glu
50 55 60
Val Leu Ala Lys Ile Ser Glu Gly Gln Val Val Thr Val Glu Arg Ile
65 70 75 80
Gln Asp Leu Val Glu Ser Gln Leu Tyr Ile Ser Gly Leu Gln Asp Val
85 90 95
Ala Arg Asp Tyr Ile Val Tyr Arg Asp Gln Arg Lys Ala Glu Arg Gly
100 105 110
Asn Ser Ser Ser Ile Ile Ala Ile Ile Arg Arg Asp Gly Gly Ser Ala
115 120 125
Lys Phe Asn Pro Met Lys Ile Ser Ala Ala Leu Glu Lys Ala Phe Arg
130 135 140
Ala Thr Leu Gln Ile Asn Gly Met Thr Pro Pro Ala Thr Leu Ser Glu
145 150 155 160
Ile Asn Asp Leu Thr Leu Arg Ile Val Glu Asp Val Leu Ser Leu His
165 170 175
Gly Glu Glu Ala Ile Asn Leu Glu Glu Ile Gln Asp Ile Val Glu Lys
180 185 190
Gln Leu Met Val Ala Gly Tyr Tyr Asp Val Ala Lys Asn Tyr Ile Leu
195 200 205
Tyr Arg Glu Ala Arg Ala Arg Ala Arg Ala Asn Lys Asp Gln Asp Gly
210 215 220
Gln Glu Glu Phe Val Pro Gln Glu Glu Thr Tyr Val Val Gln Lys Glu
225 230 235 240
Asp Gly Thr Thr Tyr Leu Leu Arg Lys Thr Asp Leu Glu Lys Arg Phe
245 250 255
Ser Trp Ala Cys Lys Arg Phe Pro Lys Thr Thr Asp Ser Gln Leu Leu
260 265 270
Ala Asp Met Ala Phe Met Asn Leu Tyr Ser Gly Ile Lys Glu Asp Glu
275 280 285
Val Thr Thr Ala Cys Ile Met Ala Ala Arg Ala Asn Ile Glu Arg Glu
290 295 300
Pro Asp Tyr Ala Phe Ile Ala Ala Glu Leu Leu Thr Ser Ser Leu Tyr
305 310 315 320
Glu Glu Thr Leu Gly Cys Ser Ser Gln Asp Pro Asn Leu Ser Glu Ile
325 330 335
His Lys Lys His Phe Lys Glu Tyr Ile Leu Asn Gly Glu Glu Tyr Arg
340 345 350
Leu Asn Pro Gln Leu Lys Asp Tyr Asp Leu Asp Ala Leu Ser Glu Val
355 360 365
Leu Asp Leu Ser Arg Asp Gln Gln Phe Ser Tyr Met Gly Val Gln Asn
370 375 380
Leu Tyr Asp Arg Tyr Phe Asn Leu His Glu Gly Arg Arg Leu Glu Thr
385 390 395 400
Ala Gln Ile Phe Trp Met Arg Val Ser Met Gly Leu Ala Leu Asn Glu
405 410 415
Gly Glu Gln Lys Asn Phe Trp Ala Ile Thr Phe Tyr Asn Leu Leu Ser
420 425 430
Thr Phe Arg Tyr Thr Pro Ala Thr Pro Thr Leu Phe Asn Ser Gly Met

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        435                 440                 445
Arg His Ser Gln Leu Ser Ser Cys Tyr Leu Ser Thr Val Lys Asp Asp
    450                 455                 460
Leu Ser His Ile Tyr Lys Val Ile Ser Asp Asn Ala Leu Leu Ser Lys
465                 470                 475                 480
Trp Ala Gly Gly Ile Gly Asn Asp Trp Thr Asp Val Arg Ala Thr Gly
                485                 490                 495
Ala Val Ile Lys Gly Thr Asn Gly Lys Ser Gln Gly Val Ile Pro Phe
            500                 505                 510
Ile Lys Val Ala Asn Asp Thr Ala Ile Ala Val Asn Gln Gly Gly Lys
        515                 520                 525
Arg Lys Gly Ala Met Cys Val Tyr Leu Glu Asn Trp His Leu Asp Tyr
    530                 535                 540
Glu Asp Phe Leu Glu Leu Arg Lys Asn Thr Gly Asp Glu Arg Arg Arg
545                 550                 555                 560
Thr His Asp Ile Asn Thr Ala Ser Trp Ile Pro Asp Leu Phe Phe Lys
                565                 570                 575
Arg Leu Glu Lys Lys Gly Met Trp Thr Leu Phe Ser Pro Asp Asp Val
            580                 585                 590
Pro Gly Leu His Glu Ala Tyr Gly Leu Glu Phe Glu Lys Leu Tyr Glu
        595                 600                 605
Glu Tyr Glu Arg Lys Val Glu Ser Gly Glu Ile Arg Leu Tyr Lys Lys
    610                 615                 620
Val Glu Ala Glu Val Leu Trp Arg Lys Met Leu Ser Met Leu Tyr Glu
625                 630                 635                 640
Thr Gly His Pro Trp Ile Thr Phe Lys Asp Pro Ser Asn Ile Arg Ser
                645                 650                 655
Asn Gln Asp His Val Gly Val Val Arg Cys Ser Asn Leu Cys Thr Glu
            660                 665                 670
Ile Leu Leu Asn Cys Ser Glu Ser Glu Thr Ala Val Cys Asn Leu Gly
        675                 680                 685
Ser Ile Asn Leu Val Glu His Ile Arg Asn Asp Lys Leu Asp Glu Glu
    690                 695                 700
Lys Leu Lys Glu Thr Ile Ser Ile Ala Ile Arg Ile Leu Asp Asn Val
705                 710                 715                 720
Ile Asp Leu Asn Phe Tyr Pro Thr Pro Glu Ala Lys Gln Ala Asn Leu
                725                 730                 735
Thr His Arg Ala Val Gly Leu Gly Val Met Gly Phe Gln Asp Val Leu
            740                 745                 750
Tyr Glu Leu Asn Ile Ser Tyr Ala Ser Gln Glu Ala Val Glu Phe Ser
        755                 760                 765
Asp Glu Cys Ser Glu Ile Ile Ala Tyr Tyr Ala Ile Leu Ala Ser Ser
    770                 775                 780
Leu Leu Ala Lys Glu Arg Gly Thr Tyr Ala Ser Tyr Ser Gly Ser Lys
785                 790                 795                 800
Trp Asp Arg Gly Tyr Leu Pro Leu Asp Thr Ile Glu Leu Leu Lys Glu
                805                 810                 815
Thr Arg Gly Glu His Asn Val Leu Val Asp Thr Ser Ser Lys Lys Asp
            820                 825                 830
Trp Thr Pro Val Arg Asp Thr Ile Gln Lys Tyr Gly Met Arg Asn Ser
        835                 840                 845
Gln Val Met Ala Ile Ala Pro Thr Ala Thr Ile Ser Asn Ile Ile Gly
    850                 855                 860
Val Thr Gln Ser Ile Glu Pro Met Tyr Lys His Leu Phe Val Lys Ser
865                 870                 875                 880
Asn Leu Ser Gly Glu Phe Thr Ile Pro Asn Thr Tyr Leu Ile Lys Lys
                885                 890                 895
Leu Lys Glu Leu Gly Leu Trp Asp Ala Glu Met Leu Asp Asp Leu Lys
            900                 905                 910
Tyr Phe Asp Gly Ser Leu Leu Glu Ile Glu Arg Ile Pro Asn His Leu
        915                 920                 925
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Lys Lys Leu Phe Leu Thr Ala Phe Glu Ile Glu Pro Glu Trp Ile Ile
    930                 935                 940
Glu Cys Thr Ser Arg Arg Gln Lys Trp Ile Asp Met Gly Val Ser Leu
945                 950                 955                 960
Asn Leu Tyr Leu Ala Glu Pro Asp Gly Lys Lys Leu Ser Asn Met Tyr
                965                 970                 975
Leu Thr Ala Trp Lys Lys Gly Leu Lys Thr Thr Tyr Tyr Leu Arg Ser
            980                 985                 990
Gln Ala Ala Thr Ser Val Glu Lys Ser Phe Ile Asp Ile Asn Lys Arg
        995                1000                1005
Gly Ile Gln Pro Arg Trp Met Lys Asn Lys Ser Ala Ser Thr Ser Ile
    1010                1015                1020
Val Val Glu Arg Lys Thr Thr Pro Val Cys Ser Met Glu Glu Gly Cys
1025                1030                1035                1040
Glu Ser Cys Gln
```

<210> 72
<211> 461
<212> PRT
<213> Chlamydia pneumoniae

<400> 72

```
Met Met Ser Ser Lys Arg Thr Ser Lys Ile Ala Val Leu Ser Ile Leu
                5                  10                  15
Leu Thr Phe Thr His Ser Ile Gly Phe Ala Asn Ala Asn Ser Ser Val
            20                  25                  30
Gly Leu Gly Thr Val Tyr Ile Thr Ser Glu Val Val Lys Lys Pro Gln
        35                  40                  45
Lys Gly Ser Glu Arg Lys Gln Ala Lys Lys Glu Pro Arg Ala Arg Lys
    50                  55                  60
Gly Tyr Leu Val Pro Ser Ser Arg Thr Leu Ser Ala Arg Ala Gln Lys
65                  70                  75                  80
Met Lys Asn Ser Ser Arg Lys Glu Ser Ser Gly Gly Cys Asn Glu Ile
                85                  90                  95
Ser Ala Asn Ser Thr Pro Arg Ser Val Lys Leu Arg Arg Asn Lys Arg
            100                 105                 110
Ala Glu Gln Lys Ala Ala Lys Gln Gly Phe Ser Ala Phe Ser Asn Leu
        115                 120                 125
Thr Leu Lys Ser Leu Leu Pro Lys Leu Pro Ser Lys Gln Lys Thr Ser
    130                 135                 140
Ile His Glu Arg Glu Lys Ala Thr Ser Arg Phe Val Asn Glu Ser Gln
145                 150                 155                 160
Leu Ser Ser Ala Arg Lys Arg Tyr Cys Thr Pro Ser Ser Ala Ala Pro
                165                 170                 175
Ser Leu Phe Leu Glu Thr Glu Ile Val Arg Ala Pro Val Glu Arg Thr
            180                 185                 190
Lys Glu Leu Gln Asp Asn Glu Ile His Ile Pro Val Val Gln Val Gln
        195                 200                 205
Thr Asn Pro Lys Glu Gln Asn Thr Lys Thr Thr Lys Gln Leu Ala Ser
    210                 215                 220
Gln Ala Ser Ile Gln Gln Ser Glu Gly Thr Glu Gln Ser Leu Arg Glu
225                 230                 235                 240
Leu Ala Gln Gly Ala Ser Leu Pro Val Leu Val Arg Ser Asn Pro Glu
                245                 250                 255
Val Ser Val Gln Arg Gln Lys Glu Glu Leu Leu Lys Glu Leu Val Ala
            260                 265                 270
Glu Arg Arg Gln Cys Lys Arg Lys Ser Val Arg Gln Ala Leu Glu Ala
        275                 280                 285
Arg Ser Leu Thr Lys Lys Val Ala Arg Gly Gly Ser Val Thr Ser Thr
```

```
    290                 295                 300
Leu Arg Tyr Asp Pro Glu Lys Ala Ala Glu Ile Lys Ser Arg Arg Asn
305                 310                 315                 320
Cys Lys Val Ser Pro Glu Ala Arg Glu Gln Lys Tyr Ser Ser Cys Lys
                325                 330                 335
Arg Asp Ala Arg Ala Asn Gly Lys Gln Asp Lys Thr Thr Pro Ser Glu
            340                 345                 350
Asp Ala Ser Gln Glu Glu Gln Gln Thr Gly Ala Gly Leu Val Arg Lys
        355                 360                 365
Thr Pro Lys Ser Gln Val Ala Ser Asn Ala Gln Asn Phe Tyr Arg Asn
    370                 375                 380
Ser Lys Asn Thr Asn Ile Asp Ser Tyr Leu Thr Ala Asn Gln Tyr Ser
385                 390                 395                 400
Cys Ser Ser Glu Glu Thr Asp Trp Pro Cys Ser Ser Cys Val Ser Lys
                405                 410                 415
Arg Arg Thr His Asn Ser Ile Ser Val Cys Thr Met Val Val Thr Val
            420                 425                 430
Ile Ala Met Ile Val Gly Ala Leu Ile Ile Ala Asn Ala Thr Glu Ser
        435                 440                 445
Gln Thr Thr Ser Asp Pro Thr Pro Pro Thr Pro Thr Pro
    450                 455                 460


<210> 73
<211> 576
<212> PRT
<213> Chlamydia pneumoniae

<400> 73
Met Thr Asp Phe Pro Thr His Phe Lys Gly Pro Lys Leu Asn Pro Ile
                5                   10                  15
Lys Val Asn Pro Asn Phe Phe Glu Arg Asn Pro Lys Val Ala Arg Val
            20                  25                  30
Leu Gln Ile Thr Ala Val Val Leu Gly Ile Ile Ala Leu Leu Ser Gly
        35                  40                  45
Ile Val Leu Ile Ile Gly Thr Pro Leu Gly Ala Pro Ile Ser Met Ile
    50                  55                  60
Leu Gly Gly Cys Leu Leu Ala Ser Gly Gly Ala Leu Phe Val Gly Gly
65                  70                  75                  80
Thr Ile Ala Thr Ile Leu Gln Ala Arg Asn Ser Tyr Lys Lys Ala Val
                85                  90                  95
Asn Gln Lys Lys Leu Ser Glu Pro Leu Met Glu Arg Pro Glu Leu Lys
            100                 105                 110
Ala Leu Asp Tyr Ser Leu Asp Leu Lys Glu Val Trp Asp Leu His His
        115                 120                 125
Ser Val Val Lys His Leu Lys Lys Leu Asp Leu Asn Leu Ser Lys Thr
    130                 135                 140
Gln Arg Glu Val Leu Asn Gln Ile Lys Ile Asp Asp Glu Gly Pro Ser
145                 150                 155                 160
Leu Gly Glu Cys Ala Ala Met Ile Ser Glu Asn Tyr Asp Ala Cys Leu
                165                 170                 175
Lys Met Leu Ala Tyr Arg Glu Glu Leu Leu Lys Glu Gln Thr Gln Tyr
            180                 185                 190
Gln Glu Thr Arg Phe Asn Gln Asn Leu Thr His Arg Asn Lys Val Leu
        195                 200                 205
Leu Ser Ile Leu Ser Arg Ile Thr Asp Asn Ile Ser Lys Ala Gly Gly
    210                 215                 220
Val Phe Ser Leu Lys Phe Ser Thr Leu Ser Ser Arg Met Ser Arg Ile
225                 230                 235                 240
His Thr Thr Thr Thr Val Ile Leu Ala Leu Ser Ala Val Val Ser Val
                245                 250                 255
```

```
Met Val Val Ala Ala Leu Ile Pro Gly Gly Ile Leu Ala Leu Pro Ile
            260                 265                 270
Leu Leu Ala Val Ala Ile Ser Ala Gly Val Ile Val Thr Gly Leu Ser
        275                 280                 285
Tyr Leu Val Arg Gln Ile Leu Ser Asn Thr Lys Arg Asn Arg Gln Asp
    290                 295                 300
Phe Tyr Lys Asp Phe Val Lys Asn Val Asp Ile Glu Leu Leu Asn Gln
305                 310                 315                 320
Thr Val Thr Leu Gln Arg Phe Leu Phe Glu Met Leu Lys Gly Val Leu
                325                 330                 335
Lys Glu Glu Glu Glu Val Ser Leu Glu Gly Gln Asp Trp Tyr Thr Gln
            340                 345                 350
Tyr Ile Thr Asn Ala Pro Ile Glu Lys Arg Leu Ile Glu Glu Ile Arg
        355                 360                 365
Val Thr Tyr Lys Glu Ile Asp Ala Gln Thr Lys Lys Met Lys Thr Asp
    370                 375                 380
Leu Glu Phe Leu Glu Asn Glu Val Arg Ser Gly Arg Leu Ser Val Ala
385                 390                 395                 400
Ser Pro Ser Glu Asp Pro Ser Glu Thr Pro Ile Phe Thr Gln Gly Lys
                405                 410                 415
Glu Phe Ala Lys Leu Arg Arg Gln Thr Ser Gln Asn Ile Ser Thr Ile
            420                 425                 430
Tyr Gly Pro Asp Asn Glu Asn Ile Asp Pro Glu Phe Ser Leu Pro Trp
        435                 440                 445
Met Pro Lys Lys Glu Glu Glu Ile Asp His Ser Leu Glu Pro Val Thr
    450                 455                 460
Lys Leu Glu Pro Gly Ser Arg Glu Glu Leu Leu Leu Val Glu Gly Val
465                 470                 475                 480
Asn Pro Thr Leu Arg Glu Leu Asn Met Arg Ile Ala Leu Leu Gln Gln
                485                 490                 495
Gln Leu Ser Ser Val Arg Lys Trp Arg His Pro Arg Gly Glu His Tyr
            500                 505                 510
Gly Asn Val Ile Tyr Ser Asp Thr Glu Leu Asp Arg Ile Gln Met Leu
        515                 520                 525
Glu Gly Ala Phe Tyr Asn His Leu Arg Glu Ala Gln Glu Glu Ile Thr
    530                 535                 540
Gln Ser Leu Gly Asp Leu Val Asp Ile Gln Asn Arg Ile Leu Gly Ile
545                 550                 555                 560
Ile Val Glu Gly Asp Ser Asp Ser Arg Thr Glu Glu Glu Pro Gln Glu
                565                 570                 575
```

<210> 74
<211> 361
<212> PRT
<213> Chlamydia pneumoniae

<400> 74

```
Met Gln Gln Thr Val Ile Val Ala Met Ser Gly Gly Val Asp Ser Ser
                5                  10                  15
Val Val Ala Tyr Leu Phe Lys Lys Phe Thr Asn Tyr Lys Val Ile Gly
            20                  25                  30
Leu Phe Met Lys Asn Trp Glu Glu Asp Ser Glu Gly Gly Leu Cys Ser
        35                  40                  45
Ser Thr Lys Asp Tyr Glu Asp Val Glu Arg Val Cys Leu Gln Leu Asp
    50                  55                  60
Ile Pro Tyr Tyr Thr Val Ser Phe Ala Lys Glu Tyr Arg Glu Arg Val
 65                  70                  75                  80
Phe Ala Arg Phe Leu Lys Glu Tyr Ser Leu Gly Tyr Thr Pro Asn Pro
                85                  90                  95
Asp Ile Leu Cys Asn Arg Glu Ile Lys Phe Asp Leu Leu Gln Lys Lys
```

```
            100                 105                 110
Val Gln Glu Leu Gly Gly Asp Tyr Leu Ala Thr Gly His Tyr Cys Arg
        115                 120                 125
Leu Asn Thr Glu Leu Gln Glu Thr Gln Leu Leu Arg Gly Cys Asp Pro
    130                 135                 140
Gln Lys Asp Gln Ser Tyr Phe Leu Ser Gly Thr Pro Lys Ser Ala Leu
145                 150                 155                 160
His Asn Val Leu Phe Pro Leu Gly Glu Met Asn Lys Thr Glu Val Arg
                165                 170                 175
Ala Ile Ala Ala Gln Ala Ala Leu Pro Thr Ala Glu Lys Lys Asp Ser
            180                 185                 190
Thr Gly Ile Cys Phe Ile Gly Lys Arg Pro Phe Lys Glu Phe Leu Glu
        195                 200                 205
Lys Phe Leu Pro Asn Lys Thr Gly Asn Val Ile Asp Trp Asp Thr Lys
    210                 215                 220
Glu Ile Val Gly Gln His Gln Gly Ala His Tyr Tyr Thr Ile Gly Gln
225                 230                 235                 240
Arg Arg Gly Leu Asp Leu Gly Gly Ser Glu Lys Pro Cys Tyr Val Val
                245                 250                 255
Gly Lys Asn Ile Glu Glu Asn Ser Ile Tyr Ile Val Arg Gly Glu Asp
            260                 265                 270
His Pro Gln Leu Tyr Leu Arg Glu Leu Thr Ala Arg Glu Leu Asn Trp
        275                 280                 285
Phe Thr Pro Pro Lys Ser Gly Cys His Cys Ser Ala Lys Val Arg Tyr
    290                 295                 300
Arg Ser Pro Asp Glu Ala Cys Thr Ile Asp Tyr Ser Ser Gly Asp Glu
305                 310                 315                 320
Val Lys Val Arg Phe Ser Gln Pro Val Lys Ala Val Thr Pro Gly Gln
                325                 330                 335
Thr Ile Ala Phe Tyr Gln Gly Asp Thr Cys Leu Gly Ser Gly Val Ile
            340                 345                 350
Asp Val Pro Met Ile Pro Ser Glu Gly
        355                 360
```

<210> 75
<211> 1609
<212> PRT
<213> Chlamydia pneumoniae

<400> 75

```
Met Val Ala Lys Lys Thr Val Arg Ser Tyr Arg Ser Ser Phe Ser His
                5                   10                  15
Ser Val Ile Val Ala Ile Leu Ser Ala Gly Ile Ala Phe Glu Ala His
            20                  25                  30
Ser Leu His Ser Ser Glu Leu Asp Leu Gly Val Phe Asn Lys Gln Phe
        35                  40                  45
Glu Glu His Ser Ala His Val Glu Glu Ala Gln Thr Ser Val Leu Lys
    50                  55                  60
Gly Ser Asp Pro Val Asn Pro Ser Gln Lys Glu Ser Glu Lys Val Leu
65                  70                  75                  80
Tyr Thr Gln Val Pro Leu Thr Gln Gly Ser Ser Gly Glu Ser Leu Asp
                85                  90                  95
Leu Ala Asp Ala Asn Phe Leu Glu His Phe Gln His Leu Phe Glu Glu
            100                 105                 110
Thr Thr Val Phe Gly Ile Asp Gln Lys Leu Val Trp Ser Asp Leu Asp
        115                 120                 125
Thr Arg Asn Phe Ser Gln Pro Thr Gln Glu Pro Asp Thr Ser Asn Ala
    130                 135                 140
Val Ser Glu Lys Ile Ser Ser Asp Thr Lys Glu Asn Arg Lys Asp Leu
145                 150                 155                 160
```

```
Glu Thr Glu Asp Pro Ser Lys Lys Ser Gly Leu Lys Glu Val Ser Ser
                165                 170                 175
Asp Leu Pro Lys Ser Pro Glu Thr Ala Val Ala Ala Ile Ser Glu Asp
            180                 185                 190
Leu Glu Ile Ser Glu Asn Ile Ser Ala Arg Asp Pro Leu Gln Gly Leu
        195                 200                 205
Ala Phe Phe Tyr Lys Asn Thr Ser Ser Gln Ser Ile Ser Glu Lys Asp
    210                 215                 220
Ser Ser Phe Gln Gly Ile Ile Phe Ser Gly Ser Gly Ala Asn Ser Gly
225                 230                 235                 240
Leu Gly Phe Glu Asn Leu Lys Ala Pro Lys Ser Gly Ala Ala Val Tyr
                245                 250                 255
Ser Asp Arg Asp Ile Val Phe Glu Asn Leu Val Lys Gly Leu Ser Phe
            260                 265                 270
Ile Ser Cys Glu Ser Leu Glu Asp Gly Ser Ala Ala Gly Val Asn Ile
        275                 280                 285
Val Val Thr His Cys Gly Asp Val Thr Leu Thr Asp Cys Ala Thr Gly
    290                 295                 300
Leu Asp Leu Glu Ala Leu Arg Leu Val Lys Asp Phe Ser Arg Gly Gly
305                 310                 315                 320
Ala Val Phe Thr Ala Arg Asn His Glu Val Gln Asn Asn Leu Ala Gly
                325                 330                 335
Gly Ile Leu Ser Val Val Gly Asn Lys Gly Ala Ile Val Val Glu Lys
            340                 345                 350
Asn Ser Ala Glu Lys Ser Asn Gly Gly Ala Phe Ala Cys Gly Ser Phe
        355                 360                 365
Val Tyr Ser Asn Asn Glu Asn Thr Ala Leu Trp Lys Glu Asn Gln Ala
    370                 375                 380
Leu Ser Gly Gly Ala Ile Ser Ser Ala Ser Asp Ile Asp Ile Gln Gly
385                 390                 395                 400
Asn Cys Ser Ala Ile Glu Phe Ser Gly Asn Gln Ser Leu Ile Ala Leu
                405                 410                 415
Gly Glu His Ile Gly Leu Thr Asp Phe Val Gly Gly Gly Ala Leu Ala
            420                 425                 430
Ala Gln Gly Thr Leu Thr Leu Arg Asn Asn Ala Val Val Gln Cys Val
        435                 440                 445
Lys Asn Thr Ser Lys Thr His Gly Gly Ala Ile Leu Ala Gly Thr Val
    450                 455                 460
Asp Leu Asn Glu Thr Ile Ser Glu Val Ala Phe Lys Gln Asn Thr Ala
465                 470                 475                 480
Ala Leu Thr Gly Gly Ala Leu Ser Ala Asn Asp Lys Val Ile Ile Ala
                485                 490                 495
Asn Asn Phe Gly Glu Ile Leu Phe Glu Gln Asn Glu Val Arg Asn His
            500                 505                 510
Gly Gly Ala Ile Tyr Cys Gly Cys Arg Ser Asn Pro Lys Leu Glu Gln
        515                 520                 525
Lys Asp Ser Gly Glu Asn Ile Asn Ile Ile Gly Asn Ser Gly Ala Ile
    530                 535                 540
Thr Phe Leu Lys Asn Lys Ala Ser Val Leu Glu Val Met Thr Gln Ala
545                 550                 555                 560
Glu Asp Tyr Ala Gly Gly Gly Ala Leu Trp Gly His Asn Val Leu Leu
                565                 570                 575
Asp Ser Asn Ser Gly Asn Ile Gln Phe Ile Gly Asn Ile Gly Gly Ser
            580                 585                 590
Thr Phe Trp Ile Gly Glu Tyr Val Gly Gly Gly Ala Ile Leu Ser Thr
        595                 600                 605
Asp Arg Val Thr Ile Ser Asn Asn Ser Gly Asp Val Val Phe Lys Gly
    610                 615                 620
Asn Lys Gly Gln Cys Leu Ala Gln Lys Tyr Val Ala Pro Gln Glu Thr
625                 630                 635                 640
Ala Pro Val Glu Ser Asp Ala Ser Ser Thr Asn Lys Asp Glu Lys Ser
```

645 650 655
Leu Asn Ala Cys Ser His Gly Asp His Tyr Pro Pro Lys Thr Val Glu
660 665 670
Glu Glu Val Pro Pro Ser Leu Leu Glu Glu His Pro Val Val Ser Ser
675 680 685
Thr Asp Ile Arg Gly Gly Gly Ala Ile Leu Ala Gln His Ile Phe Ile
690 695 700
Thr Asp Asn Thr Gly Asn Leu Arg Phe Ser Gly Asn Leu Gly Gly Gly
705 710 715 720
Glu Glu Ser Ser Thr Val Gly Asp Leu Ala Ile Val Gly Gly Gly Ala
725 730 735
Leu Leu Ser Thr Asn Glu Val Asn Val Cys Ser Asn Gln Asn Val Val
740 745 750
Phe Ser Asp Asn Val Thr Ser Asn Gly Cys Asp Ser Gly Gly Ala Ile
755 760 765
Leu Ala Lys Lys Val Asp Ile Ser Ala Asn His Ser Val Glu Phe Val
770 775 780
Ser Asn Gly Ser Gly Lys Phe Gly Gly Ala Val Cys Ala Leu Asn Glu
785 790 795 800
Ser Val Asn Ile Thr Asp Asn Gly Ser Ala Val Ser Phe Ser Lys Asn
805 810 815
Arg Thr Arg Leu Gly Gly Ala Gly Val Ala Ala Pro Gln Gly Ser Val
820 825 830
Thr Ile Cys Gly Asn Gln Gly Asn Ile Ala Phe Lys Glu Asn Phe Val
835 840 845
Phe Gly Ser Glu Asn Gln Arg Ser Gly Gly Gly Ala Ile Ile Ala Asn
850 855 860
Ser Ser Val Asn Ile Gln Asp Asn Ala Gly Asp Ile Leu Phe Val Ser
865 870 875 880
Asn Ser Thr Gly Ser Tyr Gly Gly Ala Ile Phe Val Gly Ser Leu Val
885 890 895
Ala Ser Glu Gly Ser Asn Pro Arg Thr Leu Thr Ile Thr Gly Asn Ser
900 905 910
Gly Asp Ile Leu Phe Ala Lys Asn Ser Thr Gln Thr Ala Ala Ser Leu
915 920 925
Ser Glu Lys Asp Ser Phe Gly Gly Gly Ala Ile Tyr Thr Gln Asn Leu
930 935 940
Lys Ile Val Lys Asn Ala Gly Asn Val Ser Phe Tyr Gly Asn Arg Ala
945 950 955 960
Pro Ser Gly Ala Gly Val Gln Ile Ala Asp Gly Gly Thr Val Cys Leu
965 970 975
Glu Ala Phe Gly Gly Asp Ile Leu Phe Glu Gly Asn Ile Asn Phe Asp
980 985 990
Gly Ser Phe Asn Ala Ile His Leu Cys Gly Asn Asp Ser Lys Ile Val
995 1000 1005
Glu Leu Ser Ala Val Gln Asp Lys Asn Ile Ile Phe Gln Asp Ala Ile
1010 1015 1020
Thr Tyr Glu Glu Asn Thr Ile Arg Gly Leu Pro Asp Lys Asp Val Ser
1025 1030 1035 1040
Pro Leu Ser Ala Pro Ser Leu Ile Phe Asn Ser Lys Pro Gln Asp Asp
1045 1050 1055
Ser Ala Gln His His Glu Gly Thr Ile Arg Phe Ser Arg Gly Val Ser
1060 1065 1070
Lys Ile Pro Gln Ile Ala Ala Ile Gln Glu Gly Thr Leu Ala Leu Ser
1075 1080 1085
Gln Asn Ala Glu Leu Trp Leu Ala Gly Leu Lys Gln Glu Thr Gly Ser
1090 1095 1100
Ser Ile Val Leu Ser Ala Gly Ser Ile Leu Arg Ile Phe Asp Ser Gln
1105 1110 1115 1120
Val Asp Ser Ser Ala Pro Leu Pro Thr Glu Asn Lys Glu Glu Thr Leu
1125 1130 1135

Val Ser Ala Gly Val Gln Ile Asn Met Ser Ser Pro Thr Pro Asn Lys
1140 1145 1150
Asp Lys Ala Val Asp Thr Pro Val Leu Ala Asp Ile Ile Ser Ile Thr
1155 1160 1165
Val Asp Leu Ser Ser Phe Val Pro Glu Gln Asp Gly Thr Leu Pro Leu
1170 1175 1180
Pro Pro Glu Ile Ile Ile Pro Lys Gly Thr Lys Leu His Ser Asn Ala
1185 1190 1195 1200
Ile Asp Leu Lys Ile Ile Asp Pro Thr Asn Val Gly Tyr Glu Asn His
1205 1210 1215
Ala Leu Leu Ser Ser His Lys Asp Ile Pro Leu Ile Ser Leu Lys Thr
1220 1225 1230
Ala Glu Gly Met Thr Gly Thr Pro Thr Ala Asp Ala Ser Leu Ser Asn
1235 1240 1245
Ile Lys Ile Asp Val Ser Leu Pro Ser Ile Thr Pro Ala Thr Tyr Gly
1250 1255 1260
His Thr Gly Val Trp Ser Glu Ser Lys Met Glu Asp Gly Arg Leu Val
1265 1270 1275 1280
Val Gly Trp Gln Pro Thr Gly Tyr Lys Leu Asn Pro Glu Lys Gln Gly
1285 1290 1295
Ala Leu Val Leu Asn Asn Leu Trp Ser His Tyr Thr Asp Leu Arg Ala
1300 1305 1310
Leu Lys Gln Glu Ile Phe Ala His His Thr Ile Ala Gln Arg Met Glu
1315 1320 1325
Leu Asp Phe Ser Thr Asn Val Trp Gly Ser Gly Leu Gly Val Val Glu
1330 1335 1340
Asp Cys Gln Asn Ile Gly Glu Phe Asp Gly Phe Lys His His Leu Thr
1345 1350 1355 1360
Gly Tyr Ala Leu Gly Leu Asp Thr Gln Leu Val Glu Asp Phe Leu Ile
1365 1370 1375
Gly Gly Cys Phe Ser Gln Phe Phe Gly Lys Thr Glu Ser Gln Ser Tyr
1380 1385 1390
Lys Ala Lys Asn Asp Val Lys Ser Tyr Met Gly Ala Ala Tyr Ala Gly
1395 1400 1405
Ile Leu Ala Gly Pro Trp Leu Ile Lys Gly Ala Phe Val Tyr Gly Asn
1410 1415 1420
Ile Asn Asn Asp Leu Thr Thr Asp Tyr Gly Thr Leu Gly Ile Ser Thr
1425 1430 1435 1440
Gly Ser Trp Ile Gly Lys Gly Phe Ile Ala Gly Thr Ser Ile Asp Tyr
1445 1450 1455
Arg Tyr Ile Val Asn Pro Arg Arg Phe Ile Ser Ala Ile Val Ser Thr
1460 1465 1470
Val Val Pro Phe Val Glu Ala Glu Tyr Val Arg Ile Asp Leu Pro Glu
1475 1480 1485
Ile Ser Glu Gln Gly Lys Glu Val Arg Thr Phe Gln Lys Thr Arg Phe
1490 1495 1500
Glu Asn Val Ala Ile Pro Phe Gly Phe Ala Leu Glu His Ala Tyr Ser
1505 1510 1515 1520
Arg Gly Ser Arg Ala Glu Val Asn Ser Val Gln Leu Ala Tyr Val Phe
1525 1530 1535
Asp Val Tyr Arg Lys Gly Pro Val Ser Leu Ile Thr Leu Lys Asp Ala
1540 1545 1550
Ala Tyr Ser Trp Lys Ser Tyr Gly Val Asp Ile Pro Cys Lys Ala Trp
1555 1560 1565
Lys Ala Arg Leu Ser Asn Asn Thr Glu Trp Asn Ser Tyr Leu Ser Thr
1570 1575 1580
Tyr Leu Ala Phe Asn Tyr Glu Trp Arg Glu Asp Leu Ile Ala Tyr Asp
1585 1590 1595 1600
Phe Asn Gly Gly Ile Arg Ile Ile Phe
1605

<210> 76
<211> 196
<212> PRT
<213> Chlamydia pneumoniae

<400> 76
Met Thr Leu Ser Leu Val Gly Lys Glu Ala Pro Asp Phe Val Ala Gln
1 5 10 15
Ala Val Val Asn Gly Glu Thr Cys Thr Val Ser Leu Lys Asp Tyr Leu
20 25 30
Gly Lys Tyr Val Val Leu Phe Phe Tyr Pro Lys Asp Phe Thr Tyr Val
35 40 45
Cys Pro Thr Glu Leu His Ala Phe Gln Asp Ala Leu Gly Glu Phe His
50 55 60
Thr Arg Gly Ala Glu Val Ile Gly Cys Ser Val Asp Asp Ile Ala Thr
65 70 75 80
His Gln Gln Trp Leu Ala Thr Lys Lys Lys Gln Gly Gly Ile Glu Gly
85 90 95
Ile Thr Tyr Pro Leu Leu Ser Asp Glu Asp Lys Val Ile Ser Arg Ser
100 105 110
Tyr His Val Leu Lys Pro Glu Glu Glu Leu Ser Phe Arg Gly Val Phe
115 120 125
Leu Ile Asp Lys Gly Gly Ile Ile Arg His Leu Val Val Asn Asp Leu
130 135 140
Pro Leu Gly Arg Ser Ile Glu Glu Glu Leu Arg Thr Leu Asp Ala Leu
145 150 155 160
Ile Phe Phe Glu Thr Asn Gly Leu Val Cys Pro Ala Asn Trp His Glu
165 170 175
Gly Glu Arg Ala Met Ala Pro Asn Glu Glu Gly Leu Gln Asn Tyr Phe
180 185 190
Gly Thr Ile Asp
195

<210> 77
<211> 619
<212> PRT
<213> Chlamydia pneumoniae

<400> 77
Met Lys Lys Gly Lys Leu Gly Ala Ile Val Phe Gly Leu Leu Phe Thr
1 5 10 15
Ser Ser Val Ala Gly Phe Ser Lys Asp Leu Thr Lys Asp Asn Ala Tyr
20 25 30
Gln Asp Leu Asn Val Ile Glu His Leu Ile Ser Leu Lys Tyr Ala Pro
35 40 45
Leu Pro Trp Lys Glu Leu Leu Phe Gly Trp Asp Leu Ser Gln Gln Thr
50 55 60
Gln Gln Ala Arg Leu Gln Leu Val Leu Glu Glu Lys Pro Thr Thr Asn
65 70 75 80
Tyr Cys Gln Lys Val Leu Ser Asn Tyr Val Arg Ser Leu Asn Asp Tyr
85 90 95
His Ala Gly Ile Thr Phe Tyr Arg Thr Glu Ser Ala Tyr Ile Pro Tyr
100 105 110
Val Leu Lys Leu Ser Glu Asp Gly His Val Phe Val Val Asp Val Gln
115 120 125
Thr Ser Gln Gly Asp Ile Tyr Leu Gly Asp Glu Ile Leu Glu Val Asp
130 135 140
Gly Met Gly Ile Arg Glu Ala Ile Glu Ser Leu Arg Phe Gly Arg Gly
145 150 155 160

```
Ser Ala Thr Asp Tyr Ser Ala Ala Val Arg Ser Leu Thr Ser Arg Ser
                165                 170                 175
Ala Ala Phe Gly Asp Ala Val Pro Ser Gly Ile Ala Met Leu Lys Leu
            180                 185                 190
Arg Arg Pro Ser Gly Leu Ile Arg Ser Thr Pro Val Arg Trp Arg Tyr
        195                 200                 205
Thr Pro Glu His Ile Gly Asp Phe Ser Leu Val Ala Pro Leu Ile Pro
    210                 215                 220
Glu His Lys Pro Gln Leu Pro Thr Gln Ser Cys Val Leu Phe Arg Ser
225                 230                 235                 240
Gly Val Asn Ser Gln Ser Ser Ser Ser Ser Leu Phe Ser Ser Tyr Met
                245                 250                 255
Val Pro Tyr Phe Trp Glu Glu Leu Arg Val Gln Asn Lys Gln Arg Phe
            260                 265                 270
Asp Ser Asn His His Ile Gly Ser Arg Asn Gly Phe Leu Pro Thr Phe
        275                 280                 285
Gly Pro Ile Leu Trp Glu Gln Asp Lys Gly Pro Tyr Arg Ser Tyr Ile
    290                 295                 300
Phe Lys Ala Lys Asp Ser Gln Gly Asn Pro His Arg Ile Gly Phe Leu
305                 310                 315                 320
Arg Ile Ser Ser Tyr Val Trp Thr Asp Leu Glu Gly Leu Glu Glu Asp
                325                 330                 335
His Lys Asp Ser Pro Trp Glu Leu Phe Gly Glu Ile Ile Asp His Leu
            340                 345                 350
Glu Lys Glu Thr Asp Ala Leu Ile Ile Asp Gln Thr His Asn Pro Gly
        355                 360                 365
Gly Ser Val Phe Tyr Leu Tyr Ser Leu Leu Ser Met Leu Thr Asp His
    370                 375                 380
Pro Leu Asp Thr Pro Lys His Arg Met Ile Phe Thr Gln Asp Glu Val
385                 390                 395                 400
Ser Ser Ala Leu His Trp Gln Asp Leu Leu Glu Asp Val Phe Thr Asp
                405                 410                 415
Glu Gln Ala Val Ala Val Leu Gly Glu Thr Met Glu Gly Tyr Cys Met
            420                 425                 430
Asp Met His Ala Val Ala Ser Leu Gln Asn Phe Ser Gln Ser Val Leu
        435                 440                 445
Ser Ser Trp Val Ser Gly Asp Ile Asn Leu Ser Lys Pro Met Pro Leu
    450                 455                 460
Leu Gly Phe Ala Gln Val Arg Pro His Pro Lys His Gln Tyr Thr Lys
465                 470                 475                 480
Pro Leu Phe Met Leu Ile Asp Glu Asp Asp Phe Ser Cys Gly Asp Leu
                485                 490                 495
Ala Pro Ala Ile Leu Lys Asp Asn Gly Arg Ala Thr Leu Ile Gly Lys
            500                 505                 510
Pro Thr Ala Gly Ala Gly Gly Phe Val Phe Gln Val Thr Phe Pro Asn
        515                 520                 525
Arg Ser Gly Ile Lys Gly Leu Ser Leu Thr Gly Ser Leu Ala Val Arg
    530                 535                 540
Lys Asp Gly Glu Phe Ile Glu Asn Leu Gly Val Ala Pro His Ile Asp
545                 550                 555                 560
Leu Gly Phe Thr Ser Arg Asp Leu Gln Thr Ser Arg Phe Thr Asp Tyr
                565                 570                 575
Val Glu Ala Val Lys Thr Ile Val Leu Thr Ser Leu Ser Glu Asn Ala
            580                 585                 590
Lys Lys Ser Glu Glu Gln Thr Ser Pro Gln Glu Thr Pro Glu Val Ile
        595                 600                 605
Arg Val Ser Tyr Pro Thr Thr Thr Ser Ala Ser
    610                 615
```

<210> 78

<211> 651
<212> PRT
<213> Chlamydia pneumoniae

<400> 78
Met Val Asn Pro Ile Gly Pro Gly Pro Ile Asp Glu Thr Glu Arg Thr
1 5 10 15
Pro Pro Ala Asp Leu Ser Ala Gln Gly Leu Glu Ala Ser Ala Ala Asn
20 25 30
Lys Ser Ala Glu Ala Gln Arg Ile Ala Gly Ala Glu Ala Lys Pro Lys
35 40 45
Glu Ser Lys Thr Asp Ser Val Glu Arg Trp Ser Ile Leu Arg Ser Ala
50 55 60
Val Asn Ala Leu Met Ser Leu Ala Asp Lys Leu Gly Ile Ala Ser Ser
65 70 75 80
Asn Ser Ser Ser Ser Thr Ser Arg Ser Ala Asp Val Asp Ser Thr Thr
85 90 95
Ala Thr Ala Pro Thr Pro Pro Pro Pro Thr Phe Asp Asp Tyr Lys Thr
100 105 110
Gln Ala Gln Thr Ala Tyr Asp Thr Ile Phe Thr Ser Thr Ser Leu Ala
115 120 125
Asp Ile Gln Ala Ala Leu Val Ser Leu Gln Asp Ala Val Thr Asn Ile
130 135 140
Lys Asp Thr Ala Ala Thr Asp Glu Glu Thr Ala Ile Ala Ala Glu Trp
145 150 155 160
Glu Thr Lys Asn Ala Asp Ala Val Lys Val Gly Ala Gln Ile Thr Glu
165 170 175
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195 200 205
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<212> DNA
<213> Chlamydia trachomatis serovar D

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<212> DNA
<213> Chlamydia trachomatis serovar D

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<213> Chlamydia trachomatis serovar D

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<210> 87
<211> 258
<212> DNA
<213> Chlamydia trachomatis serovar D

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<212> DNA
<213> Chlamydia trachomatis serovar D

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<211> 1689
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<213> Chlamydia trachomatis serovar D

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<212> DNA
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Val Ser Ile Ser Gly Ala Gly Glu Val Ile Phe Trp Asp Asn Ser Val
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Gly Tyr Ser Pro Leu Ser Ile Val Pro Ala Ser Thr Pro Thr Pro Pro
145                 150                 155                 160
Ala Pro Ala Pro Ala Pro Ala Ala Ser Ser Leu Ser Pro Thr Val
                165                 170                 175
Ser Asp Ala Arg Lys Gly Ser Ile Phe Ser Val Glu Thr Ser Leu Glu
            180                 185                 190
Ile Ser Gly Val Lys Lys Gly Val Met Phe Asp Asn Asn Ala Gly Asn
        195                 200                 205
Phe Gly Thr Val Phe Arg Gly Asn Ser Asn Asn Asn Ala Gly Ser Gly
    210                 215                 220
Gly Ser Gly Ser Ala Thr Thr Pro Ser Phe Thr Val Lys Asn Cys Lys
225                 230                 235                 240
Gly Lys Val Ser Phe Thr Asp Asn Val Ala Ser Cys Gly Gly Gly Val
                245                 250                 255
Val Tyr Lys Gly Thr Val Leu Phe Lys Asp Asn Glu Gly Gly Ile Phe
            260                 265                 270
Phe Arg Gly Asn Thr Ala Tyr Asp Asp Leu Gly Ile Leu Ala Ala Thr
        275                 280                 285
Ser Arg Asp Gln Asn Thr Glu Thr Gly Gly Gly Gly Gly Val Ile Cys
    290                 295                 300
Ser Pro Asp Asp Ser Val Lys Phe Glu Gly Asn Lys Gly Ser Ile Val
305                 310                 315                 320
Phe Asp Tyr Asn Phe Ala Lys Gly Arg Gly Gly Ser Ile Leu Thr Lys
                325                 330                 335
Glu Phe Ser Leu Val Ala Asp Asp Ser Val Val Phe Ser Asn Asn Thr
            340                 345                 350
Ala Glu Lys Gly Gly Gly Ala Ile Tyr Ala Pro Thr Ile Asp Ile Ser
```

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        355                 360                 365
Thr Asn Gly Gly Ser Ile Leu Phe Glu Arg Asn Arg Ala Ala Glu Gly
    370                 375                 380
Gly Ala Ile Cys Val Ser Glu Ala Ser Ser Gly Ser Thr Gly Asn Leu
385                 390                 395                 400
Thr Leu Ser Ala Ser Asp Gly Asp Ile Val Phe Ser Gly Asn Met Thr
                405                 410                 415
Ser Asp Arg Pro Gly Glu Arg Ser Ala Ala Arg Ile Leu Ser Asp Gly
            420                 425                 430
Thr Thr Val Ser Leu Asn Ala Ser Gly Leu Ser Lys Leu Ile Phe Tyr
        435                 440                 445
Asp Pro Val Val Gln Asn Asn Ser Ala Ala Gly Ala Ser Thr Pro Ser
    450                 455                 460
Pro Ser Ser Ser Met Pro Gly Ala Val Thr Ile Asn Gln Ser Gly
465                 470                 475                 480
Asn Gly Ser Val Ile Phe Thr Ala Glu Ser Leu Thr Pro Ser Glu Lys
                485                 490                 495
Leu Gln Val Leu Asn Ser Thr Ser Asn Phe Pro Gly Ala Leu Thr Val
            500                 505                 510
Ser Gly Gly Glu Leu Val Val Thr Glu Gly Ala Thr Leu Thr Thr Gly
        515                 520                 525
Thr Ile Thr Ala Thr Ser Gly Arg Val Thr Leu Gly Ser Gly Ala Ser
    530                 535                 540
Leu Ser Ala Val Ala Gly Ala Ala Asn Asn Asn Tyr Thr Cys Thr Val
545                 550                 555                 560
Ser Lys Leu Gly Ile Asp Leu Glu Ser Phe Leu Thr Pro Asn Tyr Lys
                565                 570                 575
Thr Ala Ile Leu Gly Ala Asp Gly Thr Val Thr Val Asn Ser Gly Ser
            580                 585                 590
Thr Leu Asp Leu Val Met Glu Ser Glu Ala Glu Val Tyr Asp Asn Pro
        595                 600                 605
Leu Phe Val Gly Ser Leu Thr Ile Pro Phe Val Thr Leu Ser Ser Ser
    610                 615                 620
Ser Ala Ser Asn Gly Val Thr Lys Asn Ser Val Thr Ile Asn Asp Ala
625                 630                 635                 640
Asp Ala Ala His Tyr Gly Tyr Gln Gly Ser Trp Ser Ala Asp Trp Thr
                645                 650                 655
Lys Pro Pro Leu Ala Pro Asp Ala Lys Gly Met Val Pro Pro Asn Thr
            660                 665                 670
Asn Asn Thr Leu Tyr Leu Thr Trp Arg Pro Ala Ser Asn Tyr Gly Glu
        675                 680                 685
Tyr Arg Leu Asp Pro Gln Arg Lys Gly Glu Leu Val Pro Asn Ser Leu
    690                 695                 700
Trp Val Ala Gly Ser Ala Leu Arg Thr Phe Thr Asn Gly Leu Lys Glu
705                 710                 715                 720
His Tyr Val Ser Arg Asp Val Gly Phe Val Ala Ser Leu His Ala Leu
                725                 730                 735
Gly Asp Tyr Ile Leu Asn Tyr Thr Gln Asp Asp Arg Asp Gly Phe Leu
            740                 745                 750
Ala Arg Tyr Gly Gly Phe Gln Ala Thr Ala Ala Ser His Tyr Glu Asn
        755                 760                 765
Gly Ser Ile Phe Gly Val Ala Phe Gly Gln Leu Tyr Gly Gln Thr Lys
    770                 775                 780
Ser Arg Met Tyr Tyr Ser Lys Asp Ala Gly Asn Met Thr Met Leu Ser
785                 790                 795                 800
Cys Phe Gly Arg Ser Tyr Val Asp Ile Lys Gly Thr Glu Thr Val Met
                805                 810                 815
Tyr Trp Glu Thr Ala Tyr Gly Tyr Ser Val His Arg Met His Thr Gln
            820                 825                 830
Tyr Phe Asn Asp Lys Thr Gln Lys Phe Asp His Ser Lys Cys His Trp
        835                 840                 845
```

```
His Asn Asn Asn Tyr Tyr Ala Phe Val Gly Ala Glu His Asn Phe Leu
    850                 855                 860
Glu Tyr Cys Ile Pro Thr Arg Gln Phe Ala Arg Asp Tyr Glu Leu Thr
865                 870                 875                 880
Gly Phe Met Arg Phe Glu Met Ala Gly Gly Trp Ser Ser Ser Thr Arg
                885                 890                 895
Glu Thr Gly Ser Leu Thr Arg Tyr Phe Ala Arg Gly Ser Gly His Asn
            900                 905                 910
Met Ser Leu Pro Ile Gly Ile Val Ala His Ala Val Ser His Val Arg
        915                 920                 925
Arg Ser Pro Pro Ser Lys Leu Thr Leu Asn Met Gly Tyr Arg Pro Asp
    930                 935                 940
Ile Trp Arg Val Thr Pro His Cys Asn Met Glu Ile Ile Ala Asn Gly
945                 950                 955                 960
Val Lys Thr Pro Ile Gln Gly Ser Pro Leu Ala Arg His Ala Phe Phe
                965                 970                 975
Leu Glu Val His Asp Thr Leu Tyr Ile His His Phe Gly Arg Ala Tyr
            980                 985                 990
Met Asn Tyr Ser Leu Asp Ala Arg Arg Arg Gln Thr Ala His Phe Val
        995                 1000                1005
Ser Met Gly Leu Asn Arg Ile Phe
    1010                1015


<210> 96
<211> 346
<212> PRT
<213> Chlamydia trachomatis serovar D

<400> 96
Met Gln Ala Asp Ile Leu Asp Gly Lys Gln Lys Arg Val Asn Leu Asn
                5                   10                  15
Ser Lys Arg Leu Val Asn Cys Asn Gln Val Asp Val Asn Gln Leu Val
            20                  25                  30
Pro Ile Lys Tyr Lys Trp Ala Trp Glu His Tyr Leu Asn Gly Cys Ala
        35                  40                  45
Asn Asn Trp Leu Pro Thr Glu Ile Pro Met Gly Lys Asp Ile Glu Leu
    50                  55                  60
Trp Lys Ser Asp Arg Leu Ser Glu Asp Glu Arg Arg Val Ile Leu Leu
65                  70                  75                  80
Asn Leu Gly Phe Phe Ser Thr Ala Glu Ser Leu Val Gly Asn Asn Ile
                85                  90                  95
Val Leu Ala Ile Phe Lys His Val Thr Asn Pro Glu Ala Arg Gln Tyr
            100                 105                 110
Leu Leu Arg Gln Ala Phe Glu Glu Ala Val His Thr His Thr Phe Leu
        115                 120                 125
Tyr Ile Cys Glu Ser Leu Gly Leu Asp Glu Lys Glu Ile Phe Asn Ala
    130                 135                 140
Tyr Asn Glu Arg Ala Ala Ile Lys Ala Lys Asp Asp Phe Gln Met Glu
145                 150                 155                 160
Ile Thr Gly Lys Val Leu Asp Pro Asn Phe Arg Thr Asp Ser Val Glu
                165                 170                 175
Gly Leu Gln Glu Phe Val Lys Asn Leu Val Gly Tyr Tyr Ile Ile Met
            180                 185                 190
Glu Gly Ile Phe Phe Tyr Ser Gly Phe Val Met Ile Leu Ser Phe His
        195                 200                 205
Arg Gln Asn Lys Met Ile Gly Ile Gly Glu Gln Tyr Gln Tyr Ile Leu
    210                 215                 220
Arg Asp Glu Thr Ile His Leu Asn Phe Gly Ile Asp Leu Ile Asn Gly
225                 230                 235                 240
Ile Lys Glu Glu Asn Pro Glu Ile Trp Thr Pro Glu Leu Gln Gln Glu
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                245                 250                 255
Ile Val Glu Leu Ile Lys Arg Ala Val Asp Leu Glu Ile Glu Tyr Ala
            260                 265                 270
Gln Asp Cys Leu Pro Arg Gly Ile Leu Gly Leu Arg Ala Ser Met Phe
        275                 280                 285
Ile Asp Tyr Val Gln His Ile Ala Asp Arg Arg Leu Glu Arg Ile Gly
    290                 295                 300
Leu Lys Pro Ile Tyr His Thr Lys Asn Pro Phe Pro Trp Met Ser Glu
305                 310                 315                 320
Thr Ile Asp Leu Asn Lys Glu Lys Asn Phe Phe Glu Thr Arg Val Ile
                325                 330                 335
Glu Tyr Gln His Ala Ala Ser Leu Thr Trp
            340                 345


<210> 97
<211> 1053
<212> PRT
<213> Chlamydia trachomatis serovar D

<400> 97
Met Phe Thr Arg Ile Val Met Val Asp Leu Gln Glu Lys Gln Cys Thr
                5                  10                  15
Ile Val Lys Arg Asn Gly Met Phe Val Pro Phe Asp Arg Asn Arg Ile
            20                  25                  30
Phe Gln Ala Leu Glu Ala Ala Phe Arg Asp Thr Arg Arg Ile Asp Asp
        35                  40                  45
His Met Pro Leu Pro Glu Asp Leu Glu Ser Ser Ile Arg Ser Ile Thr
    50                  55                  60
His Gln Val Val Lys Glu Val Val Gln Lys Ile Thr Asp Gly Gln Val
65                  70                  75                  80
Val Thr Val Glu Arg Ile Gln Asp Met Val Glu Ser Gln Leu Tyr Val
                85                  90                  95
Asn Gly Leu Gln Asp Val Ala Arg Asp Tyr Ile Val Tyr Arg Asp Asp
            100                 105                 110
Arg Lys Ala His Arg Lys Lys Ser Trp Gln Ser Leu Ser Val Val Arg
        115                 120                 125
Arg Cys Gly Thr Val Val His Phe Asn Pro Met Lys Ile Ser Ala Ala
    130                 135                 140
Leu Glu Lys Ala Phe Arg Ala Thr Asp Lys Thr Glu Gly Met Thr Pro
145                 150                 155                 160
Ser Ser Val Arg Glu Glu Ile Asn Ala Leu Thr Gln Asn Ile Val Ala
                165                 170                 175
Glu Ile Glu Glu Cys Cys Pro Gln Gln Asp Arg Arg Ile Asp Ile Glu
            180                 185                 190
Lys Ile Gln Asp Ile Val Glu Gln Gln Leu Met Val Val Gly His Tyr
        195                 200                 205
Ala Val Ala Lys Asn Tyr Ile Leu Tyr Arg Glu Ala Arg Ala Arg Val
    210                 215                 220
Arg Asp Asn Arg Glu Glu Asp Gly Ser Thr Glu Lys Thr Ile Ala Glu
225                 230                 235                 240
Glu Ala Val Glu Val Leu Ser Lys Asp Gly Ser Thr Tyr Thr Met Thr
                245                 250                 255
His Ser Gln Leu Leu Ala His Leu Ala Arg Ala Cys Ser Arg Phe Pro
            260                 265                 270
Glu Thr Thr Asp Ala Ala Leu Leu Thr Asp Met Ala Phe Ala Asn Phe
        275                 280                 285
Tyr Ser Gly Ile Lys Glu Ser Glu Val Val Leu Ala Cys Ile Met Ala
    290                 295                 300
Ala Arg Ala Asn Ile Glu Lys Glu Pro Asp Tyr Ala Phe Val Ala Ala
305                 310                 315                 320
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Glu Leu Leu Leu Asp Val Val Tyr Lys Glu Ala Leu Gly Lys Ser Lys
                325                 330                 335
Tyr Ala Glu Asp Leu Glu Gln Ala His Arg Asp His Phe Lys Arg Tyr
            340                 345                 350
Ile Ala Glu Gly Asp Thr Tyr Arg Leu Asn Ala Glu Leu Lys His Leu
        355                 360                 365
Phe Asp Leu Asp Ala Leu Ala Asp Ala Met Asp Leu Ser Arg Asp Leu
    370                 375                 380
Gln Phe Ser Tyr Met Gly Ile Gln Asn Leu Tyr Asp Arg Tyr Phe Asn
385                 390                 395                 400
His His Glu Gly Cys Arg Leu Glu Thr Pro Gln Ile Phe Trp Met Arg
                405                 410                 415
Val Ala Met Gly Leu Ala Leu Asn Glu Gln Asp Lys Thr Ser Trp Ala
            420                 425                 430
Ile Thr Phe Tyr Asn Leu Leu Ser Thr Phe Arg Tyr Thr Pro Ala Thr
        435                 440                 445
Pro Thr Leu Phe Asn Ser Gly Met Arg His Ser Gln Leu Ser Ser Cys
    450                 455                 460
Tyr Leu Ser Thr Val Gln Asp Asn Leu Val Asn Ile Tyr Lys Val Ile
465                 470                 475                 480
Ala Asp Asn Ala Met Leu Ser Lys Trp Ala Gly Gly Ile Gly Asn Asp
                485                 490                 495
Trp Thr Ala Ile Arg Ala Thr Gly Ala Leu Ile Lys Gly Thr Asn Gly
            500                 505                 510
Arg Ser Gln Gly Val Ile Pro Phe Ile Lys Val Thr Asn Asp Thr Ala
        515                 520                 525
Val Ala Val Asn Gln Gly Gly Lys Arg Lys Gly Ala Val Cys Val Tyr
    530                 535                 540
Leu Glu Val Trp His Leu Asp Tyr Glu Asp Phe Leu Glu Leu Arg Lys
545                 550                 555                 560
Asn Thr Gly Asp Glu Arg Arg Arg Ala His Asp Val Asn Ile Ala Ser
                565                 570                 575
Trp Ile Pro Asp Leu Phe Phe Lys Arg Leu Gln Gln Lys Gly Thr Trp
            580                 585                 590
Thr Leu Phe Ser Pro Asp Asp Val Pro Gly Leu His Asp Ala Tyr Gly
        595                 600                 605
Glu Glu Phe Glu Arg Leu Tyr Glu Glu Tyr Glu Arg Lys Val Asp Thr
    610                 615                 620
Gly Glu Ile Arg Leu Phe Lys Lys Val Glu Ala Glu Asp Leu Trp Arg
625                 630                 635                 640
Lys Met Leu Ser Met Leu Phe Glu Thr Gly His Pro Trp Met Thr Phe
                645                 650                 655
Lys Asp Pro Ser Asn Ile Arg Ser Ala Gln Asp His Lys Gly Val Val
            660                 665                 670
Arg Cys Ser Asn Leu Cys Thr Glu Ile Leu Leu Asn Cys Ser Glu Thr
        675                 680                 685
Glu Thr Ala Val Cys Asn Leu Gly Ser Ile Asn Leu Val Gln His Ile
    690                 695                 700
Val Gly Asp Gly Leu Asp Glu Glu Lys Leu Ser Glu Thr Ile Ser Ile
705                 710                 715                 720
Ala Val Arg Met Leu Asp Asn Val Ile Asp Ile Asn Phe Tyr Pro Thr
                725                 730                 735
Lys Glu Ala Lys Glu Ala Asn Phe Ala His Arg Ala Ile Gly Leu Gly
            740                 745                 750
Val Met Gly Phe Gln Asp Ala Leu Tyr Lys Leu Asp Ile Ser Tyr Ala
        755                 760                 765
Ser Gln Glu Ala Val Glu Phe Ala Asp Tyr Ser Ser Glu Leu Ile Ser
    770                 775                 780
Tyr Tyr Ala Ile Gln Ala Ser Cys Leu Leu Ala Lys Glu Arg Gly Thr
785                 790                 795                 800
Tyr Ser Ser Tyr Lys Gly Ser Lys Trp Asp Arg Gly Leu Leu Pro Ile
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805 810 815
Asp Thr Ile Gln Leu Leu Ala Asn Tyr Arg Gly Glu Ala Asn Leu Gln
820 825 830
Met Asp Thr Ser Ser Arg Lys Asp Trp Glu Pro Ile Arg Ser Leu Val
835 840 845
Lys Glu His Gly Met Arg His Cys Gln Leu Met Ala Ile Ala Pro Thr
850 855 860
Ala Thr Ile Ser Asn Ile Ile Gly Val Thr Gln Ser Ile Glu Pro Thr
865 870 875 880
Tyr Lys His Leu Phe Val Lys Ser Asn Leu Ser Gly Glu Phe Thr Ile
885 890 895
Pro Asn Val Tyr Leu Ile Glu Lys Leu Lys Lys Leu Gly Ile Trp Asp
900 905 910
Ala Asp Met Leu Asp Asp Leu Lys Tyr Phe Asp Gly Ser Leu Leu Glu
915 920 925
Ile Glu Arg Ile Pro Asp His Leu Lys His Ile Phe Leu Thr Ala Phe
930 935 940
Glu Ile Glu Pro Glu Trp Ile Ile Glu Cys Ala Ser Arg Arg Gln Lys
945 950 955 960
Trp Ile Asp Met Gly Gln Ser Leu Asn Leu Tyr Leu Ala Gln Pro Asp
965 970 975
Gly Lys Lys Leu Ser Asn Met Tyr Leu Thr Ala Trp Lys Lys Gly Leu
980 985 990
Lys Thr Thr Tyr Tyr Leu Arg Ser Ser Ser Ala Thr Thr Val Glu Lys
995 1000 1005
Ser Phe Val Asp Ile Asn Lys Arg Gly Ile Gln Pro Arg Trp Met Lys
1010 1015 1020
Asn Lys Ser Ala Ser Ala Gly Ile Ile Val Glu Arg Ala Lys Lys Ala
1025 1030 1035 1040
Pro Val Cys Ser Leu Glu Glu Gly Cys Glu Ala Cys Gln
1045 1050

<210> 98
<211> 1531
<212> PRT
<213> Chlamydia trachomatis serovar D

<400> 98
Met Ser Ser Glu Lys Asp Ile Lys Ser Thr Cys Ser Lys Phe Ser Leu
5 10 15
Ser Val Val Ala Ala Ile Leu Ala Ser Val Ser Gly Leu Ala Ser Cys
20 25 30
Val Asp Leu His Ala Gly Gly Gln Ser Val Asn Glu Leu Val Tyr Val
35 40 45
Gly Pro Gln Ala Val Leu Leu Asp Gln Ile Arg Asp Leu Phe Val
50 55 60
Gly Ser Lys Asp Ser Gln Ala Glu Gly Gln Tyr Arg Leu Ile Val Gly
65 70 75 80
Asp Pro Ser Ser Phe Gln Glu Lys Asp Ala Asp Thr Leu Pro Gly Lys
85 90 95
Val Glu Gln Ser Thr Leu Phe Ser Val Thr Asn Pro Val Val Phe Gln
100 105 110
Gly Val Asp Gln Gln Asp Gln Val Ser Ser Gln Gly Leu Ile Cys Ser
115 120 125
Phe Thr Ser Ser Asn Leu Asp Ser Pro Arg Asp Gly Glu Ser Phe Leu
130 135 140
Gly Ile Ala Phe Val Gly Asp Ser Ser Lys Ala Gly Ile Thr Leu Thr
145 150 155 160
Asp Val Lys Ala Ser Leu Ser Gly Ala Ala Leu Tyr Ser Thr Glu Asp
165 170 175

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Leu Ile Phe Glu Lys Ile Lys Gly Gly Leu Glu Phe Ala Ser Cys Ser
      .        180                 185                 190
Ser Leu Glu Gln Gly Gly Ala Cys Ala Ala Gln Ser Ile Leu Ile His
            195                 200                 205
Asp Cys Gln Gly Leu Gln Val Lys His Cys Thr Thr Ala Val Asn Ala
        210                 215                 220
Glu Gly Ser Ser Ala Asn Asp His Leu Gly Phe Gly Gly Gly Ala Phe
225                 230                 235                 240
Phe Val Thr Gly Ser Leu Ser Gly Glu Lys Ser Leu Tyr Met Pro Ala
                245                 250                 255
Gly Asp Met Val Val Ala Asn Cys Asp Gly Ala Ile Ser Phe Glu Gly
            260                 265                 270
Asn Ser Ala Asn Phe Ala Asn Gly Gly Ala Ile Ala Ala Ser Gly Lys
        275                 280                 285
Val Leu Phe Val Ala Asn Asp Lys Lys Thr Ser Phe Ile Glu Asn Arg
    290                 295                 300
Ala Leu Ser Gly Gly Ala Ile Ala Ala Ser Ser Asp Ile Ala Phe Gln
305                 310                 315                 320
Asn Cys Ala Glu Leu Val Phe Lys Gly Asn Cys Ala Ile Gly Thr Glu
                325                 330                 335
Asp Lys Gly Ser Leu Gly Gly Gly Ala Ile Ser Ser Leu Gly Thr Val
            340                 345                 350
Leu Leu Gln Gly Asn His Gly Ile Thr Cys Asp Lys Asn Glu Ser Ala
        355                 360                 365
Ser Gln Gly Gly Ala Ile Phe Gly Lys Asn Cys Gln Ile Ser Asp Asn
    370                 375                 380
Glu Gly Pro Val Val Phe Arg Asp Ser Thr Ala Cys Leu Gly Gly Gly
385                 390                 395                 400
Ala Ile Ala Ala Gln Glu Ile Val Ser Ile Gln Asn Asn Gln Ala Gly
                405                 410                 415
Ile Ser Phe Glu Gly Gly Lys Ala Ser Phe Gly Gly Gly Ile Ala Cys
            420                 425                 430
Gly Ser Phe Ser Ser Ala Gly Gly Ala Ser Val Leu Gly Thr Ile Asp
        435                 440                 445
Ile Ser Lys Asn Leu Gly Ala Ile Ser Phe Ser Arg Thr Leu Cys Thr
    450                 455                 460
Thr Ser Asp Leu Gly Gln Met Glu Tyr Gln Gly Gly Gly Ala Leu Phe
465                 470                 475                 480
Gly Glu Asn Ile Ser Leu Ser Glu Asn Ala Gly Val Leu Thr Phe Lys
                485                 490                 495
Asp Asn Ile Val Lys Thr Phe Ala Ser Asn Gly Lys Ile Leu Gly Gly
            500                 505                 510
Gly Ala Ile Leu Ala Thr Gly Lys Val Glu Ile Thr Asn Asn Ser Glu
        515                 520                 525
Gly Ile Ser Phe Thr Gly Asn Ala Arg Ala Pro Gln Ala Leu Pro Thr
    530                 535                 540
Gln Glu Glu Phe Pro Leu Phe Ser Lys Lys Glu Gly Arg Pro Leu Ser
545                 550                 555                 560
Ser Gly Tyr Ser Gly Gly Gly Ala Ile Leu Gly Arg Glu Val Ala Ile
                565                 570                 575
Leu His Asn Ala Ala Val Val Phe Glu Gln Asn Arg Leu Gln Cys Ser
            580                 585                 590
Glu Glu Glu Ala Thr Leu Leu Gly Cys Cys Gly Gly Gly Ala Val His
        595                 600                 605
Gly Met Asp Ser Thr Ser Ile Val Gly Asn Ser Ser Val Arg Phe Gly
    610                 615                 620
Asn Asn Tyr Ala Met Gly Gln Gly Val Ser Gly Gly Ala Leu Leu Ser
625                 630                 635                 640
Lys Thr Val Gln Leu Ala Gly Asn Gly Ser Val Asp Phe Ser Arg Asn
                645                 650                 655
Ile Ala Ser Leu Gly Gly Gly Ala Leu Gln Ala Ser Glu Gly Asn Cys
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660 665 670
Glu Leu Val Asp Asn Gly Tyr Val Leu Phe Arg Asp Asn Arg Gly Arg
675 680 685
Val Tyr Gly Gly Ala Ile Ser Cys Leu Arg Gly Asp Val Val Ile Ser
690 695 700
Gly Asn Lys Gly Arg Val Glu Phe Lys Asp Asn Ile Ala Thr Arg Leu
705 710 715 720
Tyr Val Glu Glu Thr Val Glu Lys Val Glu Glu Val Glu Pro Ala Pro
725 730 735
Glu Gln Lys Asp Asn Asn Glu Leu Ser Phe Leu Gly Arg Ala Glu Gln
740 745 750
Ser Phe Ile Thr Ala Ala Asn Gln Ala Leu Phe Ala Ser Glu Asp Gly
755 760 765
Asp Leu Ser Pro Glu Ser Ser Ile Ser Ser Glu Glu Leu Ala Lys Arg
770 775 780
Arg Glu Cys Ala Gly Gly Ala Ile Phe Ala Lys Arg Val Arg Ile Val
785 790 795 800
Asp Asn Gln Glu Ala Val Val Phe Ser Asn Asn Phe Ser Asp Ile Tyr
805 810 815
Gly Gly Ala Ile Phe Thr Gly Ser Leu Arg Glu Glu Asp Lys Leu Asp
820 825 830
Gly Gln Ile Pro Glu Val Leu Ile Ser Gly Asn Ala Gly Asp Val Val
835 840 845
Phe Ser Gly Asn Ser Ser Lys Arg Asp Glu His Leu Pro His Thr Gly
850 855 860
Gly Gly Ala Ile Cys Thr Gln Asn Leu Thr Ile Ser Gln Asn Thr Gly
865 870 875 880
Asn Val Leu Phe Tyr Asn Asn Val Ala Cys Ser Gly Gly Ala Val Arg
885 890 895
Ile Glu Asp His Gly Asn Val Leu Leu Glu Ala Phe Gly Gly Asp Ile
900 905 910
Val Phe Lys Gly Asn Ser Ser Phe Arg Ala Gln Gly Ser Asp Ala Ile
915 920 925
Tyr Phe Ala Gly Lys Glu Ser His Ile Thr Ala Leu Asn Ala Thr Glu
930 935 940
Gly His Ala Ile Val Phe His Asp Ala Leu Val Phe Glu Asn Leu Glu
945 950 955 960
Glu Arg Lys Ser Ala Glu Val Leu Leu Ile Asn Ser Arg Glu Asn Pro
965 970 975
Gly Tyr Thr Gly Ser Ile Arg Phe Leu Glu Ala Glu Ser Lys Val Pro
980 985 990
Gln Cys Ile His Val Gln Gln Gly Ser Leu Glu Leu Leu Asn Gly Ala
995 1000 1005
Thr Leu Cys Ser Tyr Gly Phe Lys Gln Asp Ala Gly Ala Lys Leu Val
1010 1015 1020
Leu Ala Ala Gly Ala Lys Leu Lys Ile Leu Asp Ser Gly Thr Pro Val
1025 1030 1035 1040
Gln Gln Gly His Ala Ile Ser Lys Pro Glu Ala Glu Ile Glu Ser Ser
1045 1050 1055
Ser Glu Pro Glu Gly Ala His Ser Leu Trp Ile Ala Lys Asn Ala Gln
1060 1065 1070
Thr Thr Val Pro Met Val Asp Ile His Thr Ile Ser Val Asp Leu Ala
1075 1080 1085
Ser Phe Ser Ser Ser Gln Gln Glu Gly Thr Val Glu Ala Pro Gln Val
1090 1095 1100
Ile Val Pro Gly Gly Ser Tyr Val Arg Ser Gly Glu Leu Asn Leu Glu
1105 1110 1115 1120
Leu Val Asn Thr Thr Gly Thr Gly Tyr Glu Asn His Ala Leu Leu Lys
1125 1130 1135
Asn Glu Ala Lys Val Pro Leu Met Ser Phe Val Ala Ser Gly Asp Glu
1140 1145 1150

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Ala Ser Ala Glu Ile Ser Asn Leu Ser Val Ser Asp Leu Gln Ile His
        1155                1160                1165
Val Val Thr Pro Glu Ile Glu Glu Asp Thr Tyr Gly His Met Gly Asp
    1170                1175                1180
Trp Ser Glu Ala Lys Ile Gln Asp Gly Thr Leu Val Ile Ser Trp Asn
1185                1190                1195                1200
Pro Thr Gly Tyr Arg Leu Asp Pro Gln Lys Ala Gly Ala Leu Val Phe
                1205                1210                1215
Asn Ala Leu Trp Glu Glu Gly Ala Val Leu Ser Ala Leu Lys Asn Ala
            1220                1225                1230
Arg Phe Ala His Asn Leu Thr Ala Gln Arg Met Glu Phe Asp Tyr Ser
        1235                1240                1245
Thr Asn Val Trp Gly Phe Ala Phe Gly Gly Phe Arg Thr Leu Ser Ala
    1250                1255                1260
Glu Asn Leu Val Ala Ile Asp Gly Tyr Lys Gly Ala Tyr Gly Gly Ala
1265                1270                1275                1280
Ser Ala Gly Val Asp Ile Gln Leu Met Glu Asp Phe Val Leu Gly Val
                1285                1290                1295
Ser Gly Ala Ala Phe Leu Gly Lys Met Asp Ser Gln Lys Phe Asp Ala
            1300                1305                1310
Glu Val Ser Arg Lys Gly Val Val Gly Ser Val Tyr Thr Gly Phe Leu
        1315                1320                1325
Ala Gly Ser Trp Phe Phe Lys Gly Gln Tyr Ser Leu Gly Glu Thr Gln
    1330                1335                1340
Asn Asp Met Lys Thr Arg Tyr Gly Val Leu Gly Glu Ser Ser Ala Ser
1345                1350                1355                1360
Trp Thr Ser Arg Gly Val Leu Ala Asp Ala Leu Val Glu Tyr Arg Ser
                1365                1370                1375
Leu Val Gly Pro Val Arg Pro Thr Phe Tyr Ala Leu His Phe Asn Pro
            1380                1385                1390
Tyr Val Glu Val Ser Tyr Ala Ser Met Lys Phe Pro Gly Phe Thr Glu
        1395                1400                1405
Gln Gly Arg Glu Ala Arg Ser Phe Glu Asp Ala Ser Leu Thr Asn Ile
    1410                1415                1420
Thr Ile Pro Leu Gly Met Lys Phe Glu Leu Ala Phe Ile Lys Gly Gln
1425                1430                1435                1440
Phe Ser Glu Val Asn Ser Leu Gly Ile Ser Tyr Ala Trp Glu Ala Tyr
                1445                1450                1455
Arg Lys Val Glu Gly Gly Ala Val Gln Leu Leu Glu Ala Gly Phe Asp
            1460                1465                1470
Trp Glu Gly Ala Pro Met Asp Leu Pro Arg Gln Glu Leu Arg Val Ala
        1475                1480                1485
Leu Glu Asn Asn Thr Glu Trp Ser Ser Tyr Phe Ser Thr Val Leu Gly
    1490                1495                1500
Leu Thr Ala Phe Cys Gly Gly Phe Thr Ser Thr Asp Ser Lys Leu Gly
1505                1510                1515                1520
Tyr Glu Ala Asn Thr Gly Leu Arg Leu Ile Phe
                1525                1530


<210> 99
<211> 474
<212> PRT
<213> Chlamydia trachomatis serovar D

<400> 99
Met Lys Ile Ile His Thr Ala Ile Glu Phe Ala Pro Val Ile Lys Ala
                5                  10                  15
Gly Gly Leu Gly Asp Ala Leu Tyr Gly Leu Ala Lys Ala Leu Ala Ala
            20                  25                  30
Asn His Thr Thr Glu Val Val Ile Pro Leu Tyr Pro Lys Leu Phe Thr
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```
         35                  40                  45
Leu Pro Lys Glu Gln Asp Leu Cys Ser Ile Gln Lys Leu Ser Tyr Phe
     50                  55                  60
Phe Ala Gly Glu Gln Glu Ala Thr Ala Phe Ser Tyr Phe Tyr Glu Gly
 65                  70                  75                  80
Ile Lys Val Thr Leu Phe Lys Leu Asp Thr Gln Pro Glu Leu Phe Glu
                 85                  90                  95
Asn Ala Glu Thr Ile Tyr Thr Ser Asp Asp Ala Phe Arg Phe Cys Ala
            100                 105                 110
Phe Ser Ala Ala Ala Ala Ser Tyr Ile Gln Lys Glu Gly Ala Asn Ile
        115                 120                 125
Val His Leu His Asp Trp His Thr Gly Leu Val Ala Gly Leu Leu Lys
    130                 135                 140
Gln Gln Pro Cys Ser Gln Leu Gln Lys Ile Val Leu Thr Leu His Asn
145                 150                 155                 160
Phe Gly Tyr Arg Gly Tyr Thr Thr Arg Glu Ile Leu Glu Ala Ser Ser
                165                 170                 175
Leu Asn Glu Phe Tyr Ile Ser Gln Tyr Gln Leu Phe Arg Asp Pro Gln
            180                 185                 190
Thr Cys Val Leu Leu Lys Gly Ala Leu Tyr Cys Ser Asp Phe Val Thr
        195                 200                 205
Thr Val Ser Pro Thr Tyr Ala Lys Glu Ile Leu Glu Asp Tyr Ser Asp
    210                 215                 220
Tyr Glu Ile His Asp Ala Ile Thr Ala Arg Gln His His Leu Arg Gly
225                 230                 235                 240
Ile Leu Asn Gly Ile Asp Thr Thr Ile Trp Gly Pro Glu Thr Asp Pro
                245                 250                 255
Asn Leu Ala Lys Asn Tyr Thr Lys Glu Leu Phe Glu Thr Pro Ser Ile
            260                 265                 270
Phe Phe Glu Ala Lys Ala Glu Asn Lys Lys Ala Leu Tyr Glu Arg Leu
        275                 280                 285
Gly Leu Ser Leu Glu His Ser Pro Cys Val Cys Ile Ile Ser Arg Ile
    290                 295                 300
Ala Glu Gln Lys Gly Pro His Phe Met Lys Gln Ala Ile Leu His Ala
305                 310                 315                 320
Leu Glu Asn Ala Tyr Thr Leu Ile Ile Gly Thr Cys Tyr Gly Asn
                325                 330                 335
Gln Leu His Glu Glu Phe Ala Asn Leu Gln Glu Ser Leu Ala Asn Ser
            340                 345                 350
Pro Asp Val Arg Ile Leu Leu Thr Tyr Ser Asp Val Leu Ala Arg Gln
        355                 360                 365
Ile Phe Ala Ala Ala Asp Met Ile Cys Ile Pro Ser Met Phe Glu Pro
    370                 375                 380
Cys Gly Leu Thr Gln Met Ile Gly Met Arg Tyr Gly Thr Val Pro Leu
385                 390                 395                 400
Val Arg Ala Thr Gly Gly Leu Ala Asp Thr Val Ala Asn Gly Ile Asn
                405                 410                 415
Gly Phe Ser Phe Phe Asn Pro His Asp Phe Tyr Glu Phe Arg Asn Met
            420                 425                 430
Leu Ser Glu Ala Val Thr Thr Tyr Arg Thr Asn His Asp Lys Trp Gln
        435                 440                 445
His Ile Val Arg Ala Cys Leu Asp Phe Ser Ser Asp Leu Glu Thr Ala
    450                 455                 460
Ala Asn Lys Tyr Leu Glu Ile Tyr Lys Gln
465                 470
```

<210> 100
<211> 393
<212> PRT
<213> Chlamydia trachomatis serovar D

<400> 100
Met Lys Lys Leu Leu Lys Ser Val Leu Val Phe Ala Ala Leu Ser Ser
1 5 10 15
Ala Ser Ser Leu Gln Ala Leu Pro Val Gly Asn Pro Ala Glu Pro Ser
20 25 30
Leu Met Ile Asp Gly Ile Leu Trp Glu Gly Phe Gly Gly Asp Pro Cys
35 40 45
Asp Pro Cys Ala Thr Trp Cys Asp Ala Ile Ser Met Arg Val Gly Tyr
50 55 60
Tyr Gly Asp Phe Val Phe Asp Arg Val Leu Lys Thr Asp Val Asn Lys
65 70 75 80
Glu Phe Gln Met Gly Ala Lys Pro Thr Thr Asp Thr Gly Asn Ser Ala
85 90 95
Ala Pro Ser Thr Leu Thr Ala Arg Glu Asn Pro Ala Tyr Gly Arg His
100 105 110
Met Gln Asp Ala Glu Met Phe Thr Asn Ala Ala Cys Met Ala Leu Asn
115 120 125
Ile Trp Asp Arg Phe Asp Val Phe Cys Thr Leu Gly Ala Thr Ser Gly
130 135 140
Tyr Leu Lys Gly Asn Ser Ala Ser Phe Asn Leu Val Gly Leu Phe Gly
145 150 155 160
Asp Asn Glu Asn Gln Lys Thr Val Lys Ala Glu Ser Val Pro Asn Met
165 170 175
Ser Phe Asp Gln Ser Val Val Glu Leu Tyr Thr Asp Thr Thr Phe Ala
180 185 190
Trp Ser Val Gly Ala Arg Ala Ala Leu Trp Glu Cys Gly Cys Ala Thr
195 200 205
Leu Gly Ala Ser Phe Gln Tyr Ala Gln Ser Lys Pro Lys Val Glu Glu
210 215 220
Leu Asn Val Leu Cys Asn Ala Ala Glu Phe Thr Ile Asn Lys Pro Lys
225 230 235 240
Gly Tyr Val Gly Lys Glu Phe Pro Leu Asp Leu Thr Ala Gly Thr Asp
245 250 255
Ala Ala Thr Gly Thr Lys Asp Ala Ser Ile Asp Tyr His Glu Trp Gln
260 265 270
Ala Ser Leu Ala Leu Ser Tyr Arg Leu Asn Met Phe Thr Pro Tyr Ile
275 280 285
Gly Val Lys Trp Ser Arg Ala Ser Phe Asp Ala Asp Thr Ile Arg Ile
290 295 300
Ala Gln Pro Lys Ser Ala Thr Ala Ile Phe Asp Thr Thr Thr Leu Asn
305 310 315 320
Pro Thr Ile Ala Gly Ala Gly Asp Val Lys Thr Gly Ala Glu Gly Gln
325 330 335
Leu Gly Asp Thr Met Gln Ile Val Ser Leu Gln Leu Asn Lys Met Lys
340 345 350
Ser Arg Lys Ser Cys Gly Ile Ala Val Gly Thr Thr Ile Val Asp Ala
355 360 365
Asp Lys Tyr Ala Val Thr Val Glu Thr Arg Leu Ile Asp Glu Arg Ala
370 375 380
Ala His Val Asn Ala Gln Phe Arg Phe
385 390

<210> 101
<211> 195
<212> PRT
<213> Chlamydia trachomatis serovar D

<400> 101
Met Gly Ser Leu Val Gly Arg Gln Ala Pro Asp Phe Ser Gly Lys Ala

```
                  5                  10                  15
Val Val Cys Gly Glu Glu Lys Glu Ile Ser Leu Ala Asp Phe Arg Gly
             20                  25                  30
Lys Tyr Val Val Leu Phe Phe Tyr Pro Lys Asp Phe Thr Tyr Val Cys
         35                  40                  45
Pro Thr Glu Leu His Ala Phe Gln Asp Arg Leu Val Asp Phe Glu Glu
     50                  55                  60
Arg Gly Ala Val Val Leu Gly Cys Ser Val Asp Asp Ile Glu Thr His
 65                  70                  75                  80
Ser Arg Trp Leu Ala Val Ala Arg Asn Ala Gly Gly Ile Glu Gly Thr
                 85                  90                  95
Glu Tyr Pro Leu Leu Ala Asp Pro Ser Phe Lys Ile Ser Glu Ala Phe
            100                 105                 110
Gly Val Leu Asn Pro Glu Gly Ser Leu Ala Leu Arg Ala Thr Phe Leu
        115                 120                 125
Ile Asp Lys Tyr Gly Val Val Arg His Ala Val Ile Asn Asp Leu Pre
    130                 135                 140
Leu Gly Arg Ser Ile Asp Glu Glu Leu Arg Ile Leu Asp Ser Leu Ile
145                 150                 155                 160
Phe Phe Glu Asn His Gly Met Val Cys Pro Ala Asn Trp Arg Ser Gly
                165                 170                 175
Glu Arg Gly Met Val Pro Ser Glu Glu Gly Leu Lys Glu Tyr Phe Gln
            180                 185                 190
Thr Met Asp
        195
```

<210> 102
<211> 86
<212> PRT
<213> Chlamydia trachomatis serovar D

```
<400> 102
Met Ser Gln Asn Lys Asn Ser Ala Phe Met Gln Pro Val Asn Val Ser
                  5                  10                  15
Ala Asp Leu Ala Ala Ile Val Gly Ala Gly Pro Met Pro Arg Thr Glu
             20                  25                  30
Ile Ile Lys Lys Met Trp Asp Tyr Ile Lys Lys Asn Gly Leu Gln Asp
         35                  40                  45
Pro Thr Asn Lys Arg Asn Ile Asn Pre Asp Asp Lys Leu Ala Lys Val
     50                  55                  60
Phe Gly Thr Glu Lys Pro Ile Asp Met Phe Gln Met Thr Lys Met Val
 65                  70                  75                  80
Ser Gln His Ile Ile Lys
                 85
```

<210> 103
<211> 394
<212> PRT
<213> Chlamydia trachomatis serovar D

```
<400> 103
Met Ser Lys Glu Thr Phe Gln Arg Asn Lys Pro His Ile Asn Ile Gly
                  5                  10                  15
Thr Ile Gly His Val Asp His Gly Lys Thr Thr Leu Thr Ala Ala Ile
             20                  25                  30
Thr Arg Ala Leu Ser Gly Asp Gly Leu Ala Asp Phe Arg Asp Tyr Ser
         35                  40                  45
Ser Ile Asp Asn Thr Pro Glu Glu Lys Ala Arg Gly Ile Thr Ile Asn
     50                  55                  60
```

```
Ala Ser His Val Glu Tyr Glu Thr Ala Asn Arg His Tyr Ala His Val
 65                  70                  75                  80
Asp Cys Pro Gly His Ala Asp Tyr Val Lys Asn Met Ile Thr Gly Ala
                85                  90                  95
Ala Gln Met Asp Gly Ala Ile Leu Val Val Ser Ala Thr Asp Gly Ala
            100                 105                 110
Met Pro Gln Thr Lys Glu His Ile Leu Leu Ala Arg Gln Val Gly Val
        115                 120                 125
Pro Tyr Ile Val Val Phe Leu Asn Lys Ile Asp Met Ile Ser Glu Glu
    130                 135                 140
Asp Ala Glu Leu Val Asp Leu Val Glu Met Glu Leu Val Glu Leu Leu
145                 150                 155                 160
Glu Glu Lys Gly Tyr Lys Gly Cys Pro Ile Ile Arg Gly Ser Ala Leu
                165                 170                 175
Lys Ala Leu Glu Gly Asp Ala Ala Tyr Ile Glu Lys Val Arg Glu Leu
            180                 185                 190
Met Gln Ala Val Asp Asp Asn Ile Pro Thr Pro Glu Arg Glu Ile Asp
        195                 200                 205
Lys Pro Phe Leu Met Pro Ile Glu Asp Val Phe Ser Ile Ser Gly Arg
    210                 215                 220
Gly Thr Val Val Thr Gly Arg Ile Glu Arg Gly Ile Val Lys Val Ser
225                 230                 235                 240
Asp Lys Val Gln Leu Val Gly Leu Arg Asp Thr Lys Glu Thr Ile Val
                245                 250                 255
Thr Gly Val Glu Met Phe Arg Lys Glu Leu Pro Glu Gly Arg Ala Gly
            260                 265                 270
Glu Asn Val Gly Leu Leu Leu Arg Gly Ile Gly Lys Asn Asp Val Glu
        275                 280                 285
Arg Gly Met Val Val Cys Leu Pro Asn Ser Val Lys Pro His Thr Gln
    290                 295                 300
Phe Lys Cys Ala Val Tyr Val Leu Gln Lys Glu Glu Gly Gly Arg His
305                 310                 315                 320
Lys Pro Phe Phe Thr Gly Tyr Arg Pro Gln Phe Phe Phe Arg Thr Thr
                325                 330                 335
Asp Val Thr Gly Val Val Thr Leu Pro Glu Gly Ile Glu Met Val Met
            340                 345                 350
Pro Gly Asp Asn Val Glu Phe Glu Val Gln Leu Ile Ser Pro Val Ala
        355                 360                 365
Leu Glu Glu Gly Met Arg Phe Ala Ile Arg Glu Gly Gly Arg Thr Ile
    370                 375                 380
Gly Ala Gly Thr Ile Ser Lys Ile Ile Ala
385                 390


<210> 104
<211> 82
<212> PRT
<213> Chlamydia trachomatis serovar D

<400> 104
Met Gly Gln Asp His Arg Arg Lys Phe Leu Lys Lys Val Ser Phe Val
                5                   10                  15
Lys Lys Gln Ala Ala Phe Ala Gly Asn Phe Ile Glu Glu Ile Lys Lys
            20                  25                  30
Ile Glu Trp Val Asn Lys Arg Asp Leu Lys Arg Tyr Val Lys Ile Val
        35                  40                  45
Leu Met Asn Ile Phe Gly Phe Gly Phe Ser Ile Tyr Cys Val Asp Leu
    50                  55                  60
Ala Leu Arg Lys Ser Leu Ser Leu Phe Gly Lys Val Thr Ser Phe Phe
 65                  70                  75                  80
Phe Gly
```

<210> 105
<211> 379
<212> PRT
<213> Chlamydia trachomatis serovar D

<400> 105
Met Val Ile Pro Lys Val Asp Leu Gly Glu Ser Ala Val Met Met Gly
1 5 10 15
Tyr Lys Leu Thr Ser Gln Leu Ala Met Leu Ser Ile Leu Leu Thr Phe
20 25 30
Thr His Thr Met Gly His Ala Ser Gln Met Ser Gln Thr Leu Pro Thr
35 40 45
Ile Ile Glu Ala Gln Ala Glu Glu Ala Leu Gln Ala Asp Arg Gly Val
50 55 60
Ala Gly Gln Ala Leu Lys Lys Leu Arg Lys Lys Arg Cys Ala Ser Arg
65 70 75 80
Lys Ser Ala Cys Lys Ala Ser Phe Lys Lys Lys Asp Phe Phe Ser Cys
85 90 95
Ile Thr Asn Gly Leu Phe Ser Gly Asn His Glu Gln Arg Leu Thr Ala
100 105 110
Lys Lys Glu Asn Lys Ala Arg Gly Lys Glu Pro Arg Val Val Val Gln
115 120 125
Thr Thr Lys Lys Arg Gln Ile Thr Gln Ser Glu Lys Glu Phe Phe Asp
130 135 140
Trp Leu Cys Asn Ser Lys Arg Glu Arg Lys Leu Leu Lys Lys Lys Pro
145 150 155 160
Val Asn Thr Ser Leu Ala Lys Ser Glu Glu Leu Ser Pro Lys Glu Ala
165 170 175
Ala Ile Ala Ala Ala Arg Ala Ser Leu Ser Pro Glu Glu Lys Arg Gln
180 185 190
Leu Ile Arg Glu Trp Leu Ala Glu Glu Lys Thr Ala Arg Lys Ser Gly
195 200 205
Arg Ala Ala Cys Ala Val Ser Glu Asn Leu Lys Arg Asp Gly Ser Ile
210 215 220
Thr Ser Thr Leu Arg Tyr Asp Ala Glu Lys Ala Leu Thr Thr Arg Val
225 230 235 240
Lys Arg Asn Glu Asn Ser Val Asn Ala Arg Ala Arg Gln Arg Ala Ala
245 250 255
Leu Gln Lys Ala Lys Lys Ala Lys Thr Glu Lys Pro Glu Ala Asp Glu
260 265 270
Lys Ala Ala Glu Ala Val Ala Ala Ala Pro Thr Lys Gln Ala His Lys
275 280 285
Glu Pro Glu Asn Tyr Phe Ala Ala Thr Ala Ser Thr Asn Asn Thr Asn
290 295 300
Val Met Ser Tyr Leu Asn Ala His Gln Tyr Arg Cys Asp Ser Ser Glu
305 310 315 320
Thr Asp Trp Pro Cys Ser Ser Cys Val Thr Lys Arg Arg Ala Asn Phe
325 330 335
Gly Ile Ser Val Cys Thr Met Val Val Thr Val Ile Ala Met Ile Val
340 345 350
Gly Ala Val Ile Ile Ser Asn Ala Thr Asp Ser Thr Val Ala Gly Ser
355 360 365
Ser Gly Thr Gly Gly Gly Gly Ser Thr Gln Pro
370 375

<210> 106
<211> 563

<212> PRT
<213> Chlamydia trachomatis serovar D

<400> 106
Met Val Tyr Phe Arg Ala His Gln Pro Arg His Thr Pro Lys Thr Phe
1 5 10 15
Pro Leu Glu Val His His Ser Phe Ser Asp Lys His Pro Gln Ile Ala
20 25 30
Lys Ala Met Arg Ile Thr Gly Ile Ala Leu Ala Ala Leu Ser Leu Leu
35 40 45
Ala Val Val Ala Cys Val Ile Ala Val Ser Ala Gly Gly Ala Ala Ile
50 55 60
Pro Leu Ala Val Ile Ser Gly Ile Ala Val Met Ser Gly Leu Leu Ser
65 70 75 80
Ala Ala Thr Ile Ile Cys Ser Ala Lys Lys Ala Leu Ala Gln Arg Lys
85 90 95
Gln Lys Gln Leu Glu Glu Ser Leu Pro Leu Asp Asn Ala Thr Glu His
100 105 110
Val Ser Tyr Leu Thr Ser Asp Thr Ser Tyr Phe Asn Gln Trp Glu Ser
115 120 125
Leu Gly Ala Leu Asn Lys Gln Leu Ser Gln Ile Asp Leu Thr Ile Gln
130 135 140
Ala Pro Glu Lys Lys Leu Leu Lys Glu Val Leu Gly Ser Arg Tyr Asp
145 150 155 160
Ser Ile Asn His Ser Ile Glu Glu Ile Ser Asp Arg Phe Thr Lys Met
165 170 175
Leu Ser Leu Leu Arg Leu Arg Glu His Phe Tyr Arg Gly Glu Glu Arg
180 185 190
Tyr Ala Pro Tyr Leu Ser Pro Pro Leu Leu Asn Lys Asn Arg Leu Leu
195 200 205
Thr Gln Ile Thr Ser Asn Met Ile Arg Met Leu Pro Lys Ser Gly Gly
210 215 220
Val Phe Ser Leu Lys Ala Asn Thr Leu Ser His Ala Ser Arg Thr Leu
225 230 235 240
Tyr Thr Val Leu Lys Val Ala Leu Ser Leu Gly Val Leu Ala Gly Val
245 250 255
Ala Ala Leu Ile Ile Phe Leu Pro Pro Ser Leu Pro Phe Ile Ala Val
260 265 270
Ile Gly Val Ser Ser Leu Ala Leu Gly Met Ala Ser Phe Leu Met Ile
275 280 285
Arg Gly Ile Lys Tyr Leu Leu Glu His Ser Pro Leu Asn Arg Lys Gln
290 295 300
Leu Ala Lys Asp Ile Gln Lys Thr Ile Gly Pro Asp Val Leu Ala Ser
305 310 315 320
Met Val His Tyr Gln His Gln Leu Leu Ser His Leu His Glu Thr Leu
325 330 335
Leu Asp Glu Ala Ile Thr Ala Arg Trp Ser Glu Pro Phe Phe Ile Glu
340 345 350
His Ala Asn Leu Lys Ala Lys Ile Glu Asp Leu Thr Lys Gln Tyr Asp
355 360 365
Ile Leu Asn Ala Ala Phe Asn Lys Ser Leu Gln Gln Asp Glu Ala Leu
370 375 380
Arg Ser Gln Leu Glu Lys Arg Ala Tyr Leu Phe Pro Ile Pro Asn Asn
385 390 395 400
Asp Glu Asn Ala Lys Thr Lys Glu Ser Gln Leu Leu Asp Ser Glu Asn
405 410 415
Asp Ser Asn Ser Glu Phe Gln Glu Ile Ile Asn Lys Gly Leu Glu Ala
420 425 430
Ala Asn Lys Arg Arg Ala Asp Ala Lys Ser Lys Phe Tyr Thr Glu Asp
435 440 445
Glu Thr Ser Asp Lys Ile Phe Ser Ile Trp Lys Pro Thr Lys Asn Leu

```
    450                 455                 460
Ala Leu Glu Asp Leu Trp Arg Val His Glu Ala Cys Asn Glu Glu Gln
465                 470                 475                 480
Gln Ala Leu Leu Leu Leu Glu Asp Tyr Met Ser Tyr Lys Thr Ser Glu Cys
                485                 490                 495
Gln Ala Ala Leu Gln Lys Val Ser Gln Glu Leu Lys Ala Ala Gln Lys
            500                 505                 510
Ser Phe Ala Val Leu Glu Lys His Ala Leu Asp Arg Ser Tyr Glu Ser
        515                 520                 525
Ser Val Ala Thr Met Asp Leu Ala Arg Ala Asn Gln Glu Thr His Arg
    530                 535                 540
Leu Leu Asn Ile Leu Ser Glu Leu Gln Gln Leu Ala Gln Tyr Leu Leu
545                 550                 555                 560
Asp Asn His
```

<210> 107
<211> 358
<212> PRT
<213> Chlamydia trachomatis serovar D

<400> 107

```
Met Arg Lys Thr Val Ile Val Ala Met Ser Gly Gly Val Asp Ser Ser
                  5                  10                  15
Val Val Ala Tyr Leu Leu Lys Lys Gln Gly Glu Tyr Asn Val Val Gly
             20                  25                  30
Leu Phe Met Lys Asn Trp Gly Glu Gln Asp Glu Asn Gly Glu Cys Thr
         35                  40                  45
Ala Thr Lys Asp Phe Arg Asp Val Glu Arg Ile Ala Glu Gln Leu Ser
     50                  55                  60
Ile Pro Tyr Tyr Thr Val Ser Phe Ser Lys Glu Tyr Lys Glu Arg Val
 65                  70                  75                  80
Phe Ser Arg Phe Leu Arg Glu Tyr Ala Asn Gly Tyr Thr Pro Asn Pro
                 85                  90                  95
Asp Val Leu Cys Asn Arg Glu Ile Lys Phe Asp Leu Leu Gln Lys Lys
            100                 105                 110
Val Arg Glu Leu Lys Gly Asp Phe Leu Ala Thr Gly His Tyr Cys Arg
        115                 120                 125
Gly Gly Ala Asp Gly Thr Gly Leu Ser Arg Gly Ile Asp Pro Asn Lys
    130                 135                 140
Asp Gln Ser Tyr Phe Leu Cys Gly Thr Pro Lys Asp Ala Leu Ser Asn
145                 150                 155                 160
Val Leu Phe Pro Leu Gly Gly Met Tyr Lys Thr Glu Val Arg Arg Ile
                165                 170                 175
Ala Gln Glu Ala Gly Leu Ala Thr Ala Thr Lys Lys Asp Ser Thr Gly
            180                 185                 190
Ile Cys Phe Ile Gly Lys Arg Pro Phe Lys Ser Phe Leu Glu Gln Phe
        195                 200                 205
Val Ala Asp Ser Pro Gly Asp Ile Ile Asp Phe Asp Thr Gln Gln Val
    210                 215                 220
Val Gly Arg His Glu Gly Ala His Tyr Tyr Thr Ile Gly Gln Arg Arg
225                 230                 235                 240
Gly Leu Asn Ile Gly Gly Met Glu Lys Pro Cys Tyr Val Leu Ser Lys
                245                 250                 255
Asn Met Glu Lys Asn Ile Val Tyr Ile Val Arg Gly Glu Asp His Pro
            260                 265                 270
Leu Leu Tyr Arg Gln Glu Leu Leu Ala Lys Glu Leu Asn Trp Phe Val
        275                 280                 285
Pro Leu Gln Glu Pro Met Ile Cys Ser Ala Lys Val Arg Tyr Arg Ser
    290                 295                 300
```

```
Pro Asp Glu Lys Cys Ser Val Tyr Pro Leu Glu Asp Gly Thr Val Lys
305                 310                 315                 320
Val Ile Phe Asp Val Pro Val Lys Ala Val Thr Pro Gly Gln Thr Val
                325                 330                 335
Ala Phe Tyr Gln Gly Asp Ile Cys Leu Gly Gly Gly Val Ile Glu Val
            340                 345                 350
Pro Met Ile His Gln Leu
        355
```

<210> 108
<211> 267
<212> PRT
<213> Chlamydia trachomatis serovar D

<400> 108

```
Met Ser Arg Lys Pro Ala Ser Asn Ser Ser Arg Asn Thr Lys Arg Ser
                5                  10                  15
Ser Asp Thr Ser Trp Glu Val Ile Ala Gln Asp Tyr Asn Lys Ala Val
            20                  25                  30
Asp Arg Asp Gly His Phe Tyr His Lys Glu Val Ile Leu Pro Asn Leu
        35                  40                  45
Leu Ser Lys Leu His Ile Ser Arg Ser Ser Ser Leu Val Asp Val Gly
    50                  55                  60
Cys Gly Gln Gly Ile Leu Glu Lys His Leu Pro Lys His Leu Pro Tyr
65                  70                  75                  80
Leu Gly Ile Asp Leu Ser Pro Ser Leu Leu Arg Phe Ala Lys Lys Ser
                85                  90                  95
Ala Ser Ser Lys Ser Arg Arg Phe Leu His His Asp Met Thr Gln Pro
            100                 105                 110
Val Pro Ala Asp His His Glu Gln Phe Ser His Ala Thr Ala Ile Leu
        115                 120                 125
Ser Leu Gln Asn Met Glu Ser Pro Glu Gln Ala Ile Ala His Thr Ala
    130                 135                 140
Asn Leu Leu Ala Pro Gln Gly Arg Leu Phe Ile Val Leu Asn His Pro
145                 150                 155                 160
Cys Phe Arg Ile Pro Arg Leu Ser Ser Trp Leu Tyr Asp Glu Pro Lys
                165                 170                 175
Lys Leu Leu Ser Arg Lys Ile Asp Arg Tyr Leu Ser Pro Val Ala Val
            180                 185                 190
Pro Ile Val Val His Pro Gly Glu Lys His Ser Glu Thr Thr Tyr Ser
        195                 200                 205
Phe His Phe Pro Leu Ser Tyr Trp Val Gln Ala Leu Ser Asn His Asn
    210                 215                 220
Leu Leu Ile Asp Ser Met Glu Glu Trp Ile Ser Pro Lys Lys Ser Ser
225                 230                 235                 240

Gly Lys Arg Ala Arg Ala Glu Asn Leu Cys Arg Lys Glu Phe Pro Leu
                245                 250                 255
Phe Leu Phe Ile Ser Ala Leu Lys Ile Ser Lys
            260                 265
```

<210> 109
<211> 867
<212> PRT
<213> Chlamydia trachomatis serovar D

<400> 109

```
Met Glu Lys Phe Ser Asp Ala Val Ser Glu Ala Leu Glu Lys Ala Phe
                5                  10                  15
```

```
Glu Leu Ala Lys Asn Ser Lys His Ser Tyr Val Thr Glu Asn His Leu
            20                  25                  30
Leu Lys Ser Leu Leu Gln Asn Pro Gly Ser Leu Phe Cys Leu Val Ile
        35                  40                  45
Lys Asp Val His Gly Asn Leu Gly Leu Leu Thr Ser Ala Val Asp Asp
    50                  55                  60
Ala Leu Arg Arg Glu Pro Thr Val Val Glu Gly Thr Ala Val Ala Ser
65                  70                  75                  80
Pro Ser Pro Ser Leu Gln Gln Leu Leu Leu Asn Ala His Gln Glu Ala
                85                  90                  95
Arg Ser Met Gly Asp Glu Tyr Leu Ser Gly Asp His Leu Leu Leu Ala
            100                 105                 110
Phe Trp Arg Ser Thr Lys Glu Pro Phe Ala Ser Trp Arg Lys Thr Val
        115                 120                 125
Lys Thr Thr Ser Glu Ala Leu Lys Glu Leu Ile Thr Lys Leu Arg Gln
    130                 135                 140
Gly Ser Arg Met Asp Ser Pro Ser Ala Glu Glu Asn Leu Lys Gly Leu
145                 150                 155                 160
Glu Lys Tyr Cys Lys Asn Leu Thr Val Leu Ala Arg Glu Gly Lys Leu
                165                 170                 175
Asp Pro Val Ile Gly Arg Asp Glu Glu Ile Arg Arg Thr Ile Gln Val
            180                 185                 190
Leu Ser Arg Arg Thr Lys Asn Asn Pro Met Leu Ile Gly Glu Pro Gly
        195                 200                 205
Val Gly Lys Thr Ala Ile Ala Glu Gly Leu Ala Leu Arg Ile Val Gln
    210                 215                 220
Gly Asp Val Pro Glu Ser Leu Lys Glu Lys His Leu Tyr Val Leu Asp
225                 230                 235                 240
Met Gly Ala Leu Ile Ala Gly Ala Lys Tyr Arg Gly Glu Phe Glu Glu
                245                 250                 255
Arg Leu Lys Ser Val Leu Lys Gly Val Glu Ala Ser Glu Gly Glu Cys
            260                 265                 270
Ile Leu Phe Ile Asp Glu Val His Thr Leu Val Gly Ala Gly Ala Thr
        275                 280                 285
Asp Gly Ala Met Asp Ala Ala Asn Leu Leu Lys Pro Ala Leu Ala Arg
    290                 295                 300
Gly Thr Leu His Cys Ile Gly Ala Thr Thr Leu Asn Glu Tyr Gln Lys
305                 310                 315                 320
Tyr Ile Glu Lys Asp Ala Ala Leu Glu Arg Arg Phe Gln Pro Ile Phe
                325                 330                 335
Val Thr Glu Pro Ser Leu Glu Asp Ala Val Phe Ile Leu Arg Gly Leu
            340                 345                 350
Arg Glu Lys Tyr Glu Ile Phe His Gly Val Arg Ile Thr Glu Gly Ala
        355                 360                 365
Leu Asn Ala Ala Val Val Leu Ser Tyr Arg Tyr Ile Thr Asp Arg Phe
    370                 375                 380
Leu Pro Asp Lys Ala Ile Asp Leu Ile Asp Glu Ala Ala Ser Leu Ile
385                 390                 395                 400
Arg Met Gln Ile Gly Ser Leu Pro Leu Pro Ile Asp Glu Lys Glu Arg
                405                 410                 415
Glu Leu Ser Ala Leu Ile Val Lys Gln Glu Ala Ile Lys Arg Glu Gln
            420                 425                 430
Ala Pro Ala Tyr Gln Glu Glu Ala Glu Asp Met Gln Lys Ala Ile Asp
        435                 440                 445
Arg Val Lys Glu Glu Leu Ala Ala Leu Arg Leu Arg Trp Asp Glu Glu
    450                 455                 460
Lys Gly Leu Ile Thr Gly Leu Lys Glu Lys Lys Asn Ala Leu Glu Asn
465                 470                 475                 480
Leu Lys Phe Ala Glu Glu Glu Ala Glu Arg Thr Ala Asp Tyr Asn Arg
                485                 490                 495
Val Ala Glu Leu Arg Tyr Ser Leu Ile Pro Ser Leu Glu Glu Glu Ile
```

```
            500                 505                 510
His Leu Ala Glu Glu Ala Leu Asn Gln Arg Asp Gly Arg Leu Leu Gln
        515                 520                 525
Glu Glu Val Asp Glu Arg Leu Ile Ala Gln Val Val Ala Asn Trp Thr
    530                 535                 540
Gly Ile Pro Val Gln Lys Met Leu Glu Gly Glu Ser Glu Lys Leu Leu
545                 550                 555                 560
Val Leu Glu Glu Ser Leu Glu Glu Arg Val Val Gly Gln Pro Phe Ala
                565                 570                 575
Ile Ala Ala Val Ser Asp Ser Ile Arg Ala Ala Arg Val Gly Leu Ser
            580                 585                 590
Asp Pro Gln Arg Pro Leu Gly Val Phe Leu Phe Leu Gly Pro Thr Gly
        595                 600                 605
Val Gly Lys Thr Glu Leu Ala Lys Ala Leu Ala Glu Leu Leu Phe Asn
    610                 615                 620
Lys Glu Glu Ala Met Ile Arg Phe Asp Met Thr Glu Tyr Met Glu Lys
625                 630                 635                 640
His Ser Val Ser Lys Leu Ile Gly Ser Pro Pro Gly Tyr Val Gly Tyr
                645                 650                 655
Glu Glu Gly Gly Ser Leu Ser Glu Ala Leu Arg Arg Arg Pro Tyr Ser
            660                 665                 670
Val Val Leu Phe Asp Glu Ile Glu Lys Ala Asp Lys Glu Val Phe Asn
        675                 680                 685
Ile Leu Leu Gln Ile Phe Asp Asp Gly Ile Leu Thr Asp Ser Lys Lys
    690                 695                 700
Arg Lys Val Asn Cys Lys Asn Ala Leu Phe Ile Met Thr Ser Asn Ile
705                 710                 715                 720
Gly Ser Gln Glu Leu Ala Asp Tyr Cys Thr Lys Lys Gly Thr Ile Val
                725                 730                 735
Asp Lys Glu Ala Val Leu Ser Val Val Ala Pro Ala Leu Lys Asn Tyr
            740                 745                 750
Phe Ser Pro Glu Phe Ile Asn Arg Ile Asp Asp Ile Leu Pro Phe Val
        755                 760                 765
Pro Leu Thr Thr Glu Asp Ile Val Lys Ile Val Gly Ile Gln Met Asn
    770                 775                 780
Arg Val Ala Leu Arg Leu Leu Glu Arg Lys Ile Ser Leu Thr Trp Asp
785                 790                 795                 800
Asp Ser Leu Val Leu Phe Leu Ser Glu Gln Gly Tyr Asp Ser Ala Phe
                805                 810                 815
Gly Ala Arg Pro Leu Lys Arg Leu Ile Gln Gln Lys Val Val Thr Met
            820                 825                 830
Leu Ser Lys Ala Leu Leu Lys Gly Asp Ile Lys Pro Gly Met Ala Val
        835                 840                 845
Glu Leu Thr Met Ala Lys Asp Val Val Val Phe Lys Ile Lys Thr Asn
    850                 855                 860
Pro Ala Val
865
```

<210> 110
<211> 1170
<212> DNA
<213> Chlamydia pneumoniae

<400> 110
atgaaaaaac tcttaaagtc ggcgttatta tccgccgcat ttgctggttc tgttggctcc 60
ttacaagcct tgcctgtagg gaaccattct gatccaagct tattaattga tggtacaata 120
tgggaaggtg ctgcaggaga tccttgcgat ccttgcgcta cttggtgcga cgctattagc 180
ttacgtgctg gattttacgg agactatgtt ttcgaccgta tcttaaaagt agatgcacct 240
aaaacatttt ctatgggagc caagcctact ggatccgctg ctgcaaacta tactactgcc 300
gtagatagac ctaacccggc ctacaataag catttacacg atgcagagtg gttcactaat 360

gcaggcttca ttgccttaaa catttgggat cgctttgatg ttttctgtac tttaggagct 420
tctaatggtt acattagagg aaactctaca gcgttcaatc tcgttggttt attcggagtt 480
aaaggtacta ctgtaaatgc aaatgaacta ccaaacgttt ctttaagtaa cggagttgtt 540
gaactttaca cagacacctc tttctcttgg agcgtaggcg ctcgtggagc cttatgggaa 600
tgcggttgtg caacttt ggg agctgaattc caatatgcac agtccaaacc taaagttgaa 660
gaacttaatg tgatctgtaa cgtatcgcaa ttctctgtaa acaaacccaa gggctataaa 720
ggcgttgctt tcccctttgcc aacagacgct ggcgtagcaa cagctactgg aacaaagtct 780
gcgaccatca attatcatga atggcaagta ggagcctctc tatcttacag actaaactct 840
ttagtgccat acattggagt acaatggtct cgagcaactt ttgatgctga taacatccgc 900
attgctcagc caaaactacc tacagctgtt ttaaacttaa ctgcatggaa ccctctctta 960
ctaggaaatg ccacagcatt gtctactact gattcgttct cagacttcat gcaaattgtt 1020
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<213> Chlamydia pneumoniae

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35 40 45
Cys Asp Pro Cys Ala Thr Trp Cys Asp Ala Ile Ser Leu Arg Ala Gly
50 55 60
Phe Tyr Gly Asp Tyr Val Phe Asp Arg Ile Leu Lys Val Asp Ala Pro
65 70 75 80
Lys Thr Phe Ser Met Gly Ala Lys Pro Thr Gly Ser Ala Ala Ala Asn
85 90 95
Tyr Thr Thr Ala Val Asp Arg Pro Asn Pro Ala Tyr Asn Lys His Leu
100 105 110
His Asp Ala Glu Trp Phe Thr Asn Ala Gly Phe Ile Ala Leu Asn Ile
115 120 125
Trp Asp Arg Phe Asp Val Phe Cys Thr Leu Gly Ala Ser Asn Gly Tyr
130 135 140
Ile Arg Gly Asn Ser Thr Ala Phe Asn Leu Val Gly Leu Phe Gly Val
145 150 155 160
Lys Gly Thr Thr Val Asn Ala Asn Glu Leu Pro Asn Val Ser Leu Ser
165 170 175
Asn Gly Val Val Glu Leu Tyr Thr Asp Thr Ser Phe Ser Trp Ser Val
180 185 190
Gly Ala Arg Gly Ala Leu Trp Glu Cys Gly Cys Ala Thr Leu Gly Ala
195 200 205
Glu Phe Gln Tyr Ala Gln Ser Lys Pro Lys Val Glu Glu Leu Asn Val
210 215 220
Ile Cys Asn Val Ser Gln Phe Ser Val Asn Lys Pro Lys Gly Tyr Lys
225 230 235 240
Gly Val Ala Phe Pro Leu Pro Thr Asp Ala Gly Val Ala Thr Ala Thr
245 250 255
Gly Thr Lys Ser Ala Thr Ile Asn Tyr His Glu Trp Gln Val Gly Ala
260 265 270
Ser Leu Ser Tyr Arg Leu Asn Ser Leu Val Pro Tyr Ile Gly Val Gln
275 280 285
Trp Ser Arg Ala Thr Phe Asp Ala Asp Asn Ile Arg Ile Ala Gln Pro
290 295 300
Lys Leu Pro Thr Ala Val Leu Asn Leu Thr Ala Trp Asn Pro Ser Leu
305 310 315 320
Leu Gly Asn Ala Thr Ala Leu Ser Thr Thr Asp Ser Phe Ser Asp Phe
325 330 335
Met Gln Ile Val Ser Cys Gln Ile Asn Lys Phe Lys Ser Arg Lys Ala
340 345 350
Cys Gly Val Thr Val Gly Ala Thr Leu Val Asp Ala Asp Lys Trp Ser
355 360 365
Leu Thr Ala Glu Ala Arg Leu Ile Asn Glu Arg Ala Ala His Val Ser
370 375 380
Gly Gln Phe Arg Phe

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<213> Chlamydia pneumoniae

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35 40 45
Lys Asp Ile His Gly Asn Pro Gly Leu Leu Asn Thr Ala Val Lys Asp
50 55 60
Ala Leu Ser Arg Glu Pro Thr Val Val Glu Gly Glu Val Asp Pro Lys
65 70 75 80
Pro Ser Pro Gly Leu Gln Thr Leu Leu Arg Asp Ala Lys Gln Glu Ala
85 90 95
Lys Thr Leu Gly Asp Glu Tyr Ile Ser Gly Asp His Leu Leu Leu Ala
100 105 110
Phe Trp Ser Ser Asn Lys Glu Pro Phe Asn Ser Trp Lys Gln Thr Thr
115 120 125
Lys Val Ser Phe Lys Asp Leu Lys Asn Leu Ile Thr Lys Ile Arg Arg
130 135 140
Gly Asn Arg Met Asp Ser Pro Ser Ala Glu Ser Asn Phe Gln Gly Leu
145 150 155 160
Glu Lys Tyr Cys Lys Asn Leu Thr Ala Leu Ala Arg Glu Gly Lys Leu
165 170 175
Asp Pro Val Ile Gly Arg Asp Glu Glu Ile Arg Arg Thr Ile Gln Val
180 185 190
Leu Ser Arg Arg Thr Lys Asn Asn Pro Met Leu Ile Gly Glu Pro Gly
195 200 205
Val Gly Lys Thr Ala Ile Ala Glu Gly Leu Ala Leu Arg Leu Ile Gln
210 215 220
Gly Asp Val Pro Glu Ser Leu Lys Gly Lys Gln Leu Tyr Val Leu Asp
225 230 235 240
Met Gly Ala Leu Ile Ala Gly Ala Lys Tyr Arg Gly Glu Phe Glu Glu
245 250 255
Arg Leu Lys Ser Val Leu Lys Asp Val Glu Ser Gly Asp Gly Glu His
260 265 270
Ile Ile Phe Ile Asp Glu Val His Thr Leu Val Gly Ala Gly Ala Thr
275 280 285
Asp Gly Ala Met Asp Ala Ala Asn Leu Leu Lys Pro Ala Leu Ala Arg
290 295 300
Gly Thr Leu His Cys Ile Gly Ala Thr Thr Leu Asn Glu Tyr Gln Lys
305 310 315 320
Tyr Ile Glu Lys Asp Ala Ala Leu Glu Arg Arg Phe Gln Pro Ile Phe
325 330 335
Val Thr Glu Pro Ser Leu Glu Asp Ala Val Phe Ile Leu Arg Gly Leu
340 345 350
Arg Glu Lys Tyr Glu Ile Phe His Gly Val Arg Ile Thr Glu Gly Ala
355 360 365
Leu Asn Ala Ala Val Leu Leu Ser Tyr Arg Tyr Ile Pro Asp Arg Phe
370 375 380
Leu Pro Asp Lys Ala Ile Asp Leu Ile Asp Glu Ala Ala Ser Leu Ile
385 390 395 400
Arg Met Gln Ile Gly Ser Leu Pro Leu Pro Ile Asp Glu Lys Glu Arg
405 410 415

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Lys Lys Leu Ile Ser Gly Leu Lys Glu Lys Lys Asn Ser Leu Glu Ser
465                 470                 475                 480
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                485                 490                 495
Val Ala Glu Leu Arg Tyr Ser Leu Ile Pro Gln Leu Glu Glu Glu Ile
            500                 505                 510
Lys Gln Asp Glu Ala Ser Leu Asn Gln Arg Asp Asn Arg Leu Leu Gln
        515                 520                 525
Glu Glu Val Asp Glu Arg Leu Ile Ala Gln Val Val Ala Asn Trp Thr
    530                 535                 540
Gly Ile Pro Val Gln Lys Met Leu Glu Gly Glu Ala Glu Lys Leu Leu
545                 550                 555                 560
Ile Leu Glu Glu Ser Leu Glu Glu Arg Val Val Gly Gln Pro Phe Ala
                565                 570                 575
Val Ser Ala Val Ser Asp Ser Ile Arg Ala Ala Arg Val Gly Leu Asn
            580                 585                 590
Asp Pro Gln Arg Pro Leu Gly Val Phe Leu Phe Leu Gly Pro Thr Gly
        595                 600                 605
Val Gly Lys Thr Glu Leu Ala Lys Ala Leu Ala Asp Leu Leu Phe Asn
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Lys Glu Glu Ala Met Val Arg Phe Asp Met Ser Glu Tyr Met Glu Lys
625                 630                 635                 640
His Ser Ile Ser Lys Leu Ile Gly Ser Ser Pro Gly Tyr Val Gly Tyr
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Glu Glu Gly Gly Ser Leu Ser Glu Ala Leu Arg Arg Arg Pro Tyr Ser
            660                 665                 670
Val Val Leu Phe Asp Glu Ile Glu Lys Ala Asp Lys Glu Val Leu Asn
        675                 680                 685
Ile Leu Leu Gln Val Phe Asp Asp Gly Ile Leu Thr Asp Gly Lys Lys
    690                 695                 700
Arg Lys Val Asn Cys Lys Asn Ala Leu Phe Ile Met Thr Ser Asn Ile
705                 710                 715                 720
Gly Ser Pro Glu Leu Ala Asp Tyr Cys Ser Lys Lys Gly Ser Glu Leu
                725                 730                 735
Thr Lys Glu Ala Ile Leu Ser Val Val Ser Pro Val Leu Lys Arg Tyr
            740                 745                 750
Leu Ser Pro Glu Phe Met Asn Arg Ile Asp Glu Ile Leu Pro Phe Val
        755                 760                 765
Pro Leu Thr Lys Glu Asp Ile Val Lys Ile Val Gly Ile Gln Met Arg
    770                 775                 780
Arg Ile Ala Gln Arg Leu Lys Ala Arg Arg Ile Asn Leu Ser Trp Asp
785                 790                 795                 800
Asp Ser Val Ile Leu Phe Leu Ser Glu Gln Gly Tyr Asp Ser Ala Phe
                805                 810                 815
Gly Ala Arg Pro Leu Lys Arg Leu Ile Gln Gln Lys Val Val Ile Leu
            820                 825                 830
Leu Ser Lys Ala Leu Leu Lys Gly Asp Ile Lys Pro Asp Thr Ser Ile
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<213> Homo sapiens

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<211> 772
<212> DNA
<213> Homo sapiens

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<211> 487
<212> DNA
<213> Homo sapiens

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gatctta 487

<210> 117
<211> 1014
<212> DNA
<213> Homo sapiens

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<211> 287
<212> DNA
<213> Homo sapiens

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tccaacaaaa tttgcttaaa gcagaaaaag gaaataaagc cgcagcacaa agagttcgta 240
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<210> 119
<211> 1002
<212> DNA
<213> Homo sapiens

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<212> DNA
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agaaacctta tgcctacgcc taaagccgga actgtaacaa cagatgtggt taaaactatt 480
gcggaactgc gaaaaggtaa aattgaattt aaagctgatc gagctggtgt atgcaacgtc 540
ggagttgcga agctttcttt cgatagtgcg caaatcaaag aaaatgttga agcgttgtgt 600
gcagccttag ttaaagctaa gcccgcaact gctaaaggac aatatttagt taatttcact 660
atttcctcga ccatggggcc aggggttacc gtggatacta gggagttgat tgcgttataa 720
gaattc 726

<210> 122
<211> 330
<212> PRT
<213> Homo sapiens

<400> 122
Met His His His His His His Met Ser Ile Arg Pro Thr Asn Gly Ser

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                  5                  10                  15
Gly Asn Gly Tyr Pro Ser Ile Asn Pro Ser Asn Asp Asn Gln Tyr Gly
             20                  25                  30
Leu Val Gln Ser Thr Ser Gly Pro Asn Tyr Gly Gly His Thr Val Ser
         35                  40                  45
Ser Arg Gly Gly Phe Gln Gly Ile Cys Val Arg Ile Ala Asp Leu Phe
     50                  55                  60
Arg Asn Cys Phe Ser Arg Asn Arg Gly Thr Thr Thr Pro Ser Arg
 65                  70                  75                  80
Thr Val Ile Thr Gln Ala Asp Ile Tyr His Pro Thr Ile Ser Gly Gln
                 85                  90                  95
Gly Ala Gln Pro Ile Val Ser Thr Gly Asp Lys Lys Leu Asp Ser Ala
            100                 105                 110
Ile Ile Gln Ala Asp Leu Arg Ala Gln Asn Lys Gln Thr Leu Ala Thr
        115                 120                 125
His Ile Gln Ser Lys Leu Gly Ser Met Glu Gly Gln Ser Pro Gln Asp
    130                 135                 140
Tyr Lys Ala Gly Ala Tyr Ser Ala Leu Arg Leu Met Leu Phe Thr Pro
145                 150                 155                 160
Gly Glu Thr Thr Val Ser Ser Glu Arg Glu Arg Gln Ala Cys Val Thr
                165                 170                 175
Gly Arg Asp Leu Trp Glu Gln Ala Ala Gly Asp Leu Ala Thr Asn Gly
            180                 185                 190
Asn Thr Asp Gly Leu Met Leu Met Ala Asn Leu Ser Val Gly Gly Lys
        195                 200                 205
His Val Pro Ala Gly His Leu Arg Glu Tyr Met Asp Thr Val Lys Gly
    210                 215                 220
Thr Phe Thr Asp Glu Asn Glu Ala Thr Asp Pro Thr Val Asp Ala Ile
225                 230                 235                 240
Leu Asp Leu Ala Ala Lys Ile Asp Ala Thr Glu Phe Ser Ser Pro Gly
                245                 250                 255
Ser Gly Gln Val Ile Leu Asn Tyr Ile Gly Asn Tyr Gly Gln Val Val
            260                 265                 270
Leu Glu Asn Glu Glu Met Asn Leu Leu Val Leu Glu Asp Gln Asn Gly
        275                 280                 285
Gln Asp Pro Gln Arg Val Gln Asp Asn Ser Lys Glu Leu Gln Lys Leu
    290                 295                 300
Leu Glu Asn Ala Arg Lys Thr Asp Pro Glu Leu Tyr Phe Gln Thr Leu
305                 310                 315                 320
Thr Val Ile Thr Ser Ser Val Phe Leu Asp
                325                 330


<210> 123
<211> 405
<212> PRT
<213> Homo sapiens

<400> 123
Met His His His His His His Val Ser Ser Ile Ser Pro Ile Gly Gly
                  5                  10                  15
Asn Ser Gly Pro Glu Gly Phe Ser Ser Ala Ser Arg Gly Asp Glu Ile
             20                  25                  30
Asp Asp Val Pro Asp Ser Glu Glu Gly Glu Leu Glu Glu Arg Val Ser
         35                  40                  45
Asp His Ala Glu Ser Ile Ile Thr Glu Ser Ser Glu Thr Leu Phe Arg
     50                  55                  60
Thr Thr Ser Ser Ser Gly Val Ser Glu Asp Leu Gln Gln His Val Ser
 65                  70                  75                  80
Leu Glu Glu Ser Pro Arg Gln Arg Gly Phe Leu Gly Arg Ile Arg Asp
                 85                  90                  95
```

```
Ala Val Ala Ser Ile Trp Lys Arg Arg Val Ala Arg Arg Asn Glu Asn
            100                 105                 110
Tyr Asp Val Lys Lys Ala Glu Glu Gln Gln Gly Ile Val Gln Tyr Leu
        115                 120                 125
Gln Asp Ser Lys Met Pro Ala Leu Thr Arg Ala Tyr Arg His Leu Arg
    130                 135                 140
Ala Phe Asn Ser Ala Cys Leu Arg Thr Ile Arg Glu Phe Phe Ala Thr
145                 150                 155                 160
Ile Phe Arg Ala Leu Arg Asp Ala Tyr Tyr Arg His Cys Thr Arg Ser
                165                 170                 175
Gly Ile Asn Phe Cys Gly Ala Asp Lys Asp Ser Leu Glu Val Leu Val
            180                 185                 190
Ala Val Gly Leu Leu Leu Arg Met Ala Thr Leu Arg Ser Phe Glu His
        195                 200                 205
Val Gly Gly Asn Tyr Glu Asp Arg Leu Val Asn Asn Asp Ala Pro Val
    210                 215                 220
Thr Gly Ala Gly Arg Thr Leu Val Asp Asp Ala Val Asp Asp Ile Glu
225                 230                 235                 240
Ser Ile Leu Asn Thr Arg Thr Asn Trp Pro Gln His Val Met Ile Gly
                245                 250                 255
Phe Ser Arg Gly Leu Val Gln Leu Cys Ala Thr Pro Tyr Asn Ala Thr
            260                 265                 270
Ser Gln Glu Cys Phe Lys Ser Ile Val Arg Leu Glu Lys Glu Asp Pro
        275                 280                 285
Ser Ser Asp Tyr Ser Gln Ala Leu Leu Leu Ala Gly Ile Ile Asp Arg
    290                 295                 300
Leu Ala Glu Lys Ala Pro Met Ala Ala Lys Tyr Val Leu Asp Ala Leu
305                 310                 315                 320
Arg Val Arg Thr Ser Glu Leu Ile Gly Glu Leu Ile Ile Leu Asp Leu
                325                 330                 335
Leu Pro Pro Val Trp Lys Val Gly Arg Gly Gly Val Phe Pro Pro Val
            340                 345                 350
Asn Glu Gln Leu Val Val Gln Ile Val Asn Ala Asn Val Glu Arg Leu
        355                 360                 365
His Ser Thr Phe Ala His Glu Pro Gln Ala Tyr Leu Arg Met Ile Glu
    370                 375                 380
Gly Leu Val Thr Asn Phe Phe Phe Leu Pro Ser Glu Glu Asp Pro Ser
385                 390                 395                 400
Ser Val Gly Asn Ile
                405


<210> 124
<211> 238
<212> PRT
<213> Homo sapiens

<400> 124
Met His His His His His His Thr Lys His Gly Lys Arg Ile Arg Gly
                  5                  10                  15
Ile Gln Glu Thr Tyr Asp Leu Ala Lys Ser Tyr Ser Leu Gly Glu Ala
             20                  25                  30
Ile Asp Ile Leu Lys Gln Cys Pro Thr Val Arg Phe Asp Gln Thr Val
         35                  40                  45
Asp Val Ser Val Lys Leu Gly Ile Asp Pro Arg Lys Ser Asp Gln Gln
     50                  55                  60
Ile Arg Gly Ser Val Ser Leu Pro His Gly Thr Gly Lys Val Leu Arg
 65                  70                  75                  80
Ile Leu Val Phe Ala Ala Gly Asp Lys Ala Ala Glu Ala Ile Glu Ala
                 85                  90                  95
Gly Ala Asp Phe Val Gly Ser Asp Asp Leu Val Glu Lys Ile Lys Gly
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```
            100                 105                 110
Gly Trp Val Asp Phe Asp Val Ala Val Ala Thr Pro Asp Met Met Arg
        115                 120                 125
Glu Val Gly Lys Leu Gly Lys Val Leu Gly Pro Arg Asn Leu Met Pro
    130                 135                 140
Thr Pro Lys Ala Gly Thr Val Thr Thr Asp Val Val Lys Thr Ile Ala
145                 150                 155                 160
Glu Leu Arg Lys Gly Lys Ile Glu Phe Lys Ala Asp Arg Ala Gly Val
                165                 170                 175
Cys Asn Val Gly Val Ala Lys Leu Ser Phe Asp Ser Ala Gln Ile Lys
            180                 185                 190
Glu Asn Val Glu Ala Leu Cys Ala Ala Leu Val Lys Ala Lys Pro Ala
        195                 200                 205
Thr Ala Lys Gly Gln Tyr Leu Val Asn Phe Thr Ile Ser Ser Thr Met
    210                 215                 220
Gly Pro Gly Val Thr Val Asp Thr Arg Glu Leu Ile Ala Leu
225                 230                 235


<210> 125
<211> 713
<212> DNA
<213> Chlamydia trachomatis

<400> 125
ataacaatcc ctcccaatca tcgttgaacg tacaaggagg agccatctat gccaaaacct 60
ctttgtctat tggatcttcc gatgctggaa cctcctatat tttctcgggg aacagtgtct 120
ccactgggaa atctcaaaca acagggcaaa tagcgggagg agcgatctac tcccctactg 180
ttacattgaa ttgtcctgcg acattctcta acaatacagc ctctatagct acaccgaaga 240
cttcttctga agatggatcc tcaggaaatt ctattaaaga taccattgga ggagcccattg 300
cagggacagc cattacccta tctggagtct ctcgattttc agggaatacg gctgatttag 360
gagctgcaat aggaactcta gctaatgcaa atacacccag tgcaactagc ggatctcaaa 420
atagcattac agaaaaaatt actttagaaa acggttcttt tatttttgaa agaaaccaag 480
ctaataaacg tggagcgatt tactctccta gcgtttccat taaagggaat aatattacct 540
tcaatcaaaa tacatccact catgatggaa gcgctatcta ctttacaaaa gatgctacga 600
ttgagtcttt aggatctgtt ctttttacag gaaataacgt tacagctaca caagctagtt 660
ctgcaacatc tggacaaaat acaaatactg ccaactatgg ggcagccatc ttt         713

<210> 126
<211> 780
<212> DNA
<213> Chlamydia trachomatis

<400> 126
ccttctcctt actcaggagt tttaaaagaa aacgcaccgt ttttacgttt cctcacacaa 60
ttaactaaca agcatactca ttctggattt catgcctcc taaaattctt agtcaaatcc 120
gaaagaagcc gacactcgag cgctcttctc ctaaaaatct tgtttttct ctgcttccga 180
gttataaacgc ggctgtctca taacccacac taacatgatg aaacctctac gttttcggtta 240
tttcttttgc acaatctatt ttactttgtt acaggcagcg tttgctaaag aaccgaattc 300
ttgtcccgac tgccagaata attggaaaga agtcacccac acggatcaac tccctgaaaa 360
catcattcat gctgatgatg cttgttatca ctctggttat gtacaggctc tcattgatat 420
gcatttctta gatagctgct gccaggtcat cgttgaaaac caaactgctt acttattttc 480
tcttcctaca gatgatgtta cgcgcaacgc cattatcaac ctaattaaag accttccatt 540
cattcactcc gtagaaatct gccaagcatc ctatcaaacc tgtcatcatc aaggccctca 600
tggaaagact tctcttccag aacaacgttc tttctgtaca aaggtctgtg gaaaagaagc 660
tatttggtta ccacagaata ccatcctatt ctcgcctctt gtagcagata ctatccaagc 720
aactaatagt gcaggtatcc gttttaacga cgaagtcgta ggaaaacgtg ttggctctgc 780


<210> 127
<211> 433
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<212> DNA
<213> Chlamydia trachomatis

<400> 127
ctttaaagat tcgtcgtcct tttggtacta cgagagaagt tcgtgtgaaa tggcgttatg 60
ttcctgaagg tgtaggagat ttggctacca tagctcottc tatcagggct ccacagttac 120
agaaatcgat gagaagcttt ttccctaaga aagatgatgc gtttcatcgg tctagttcgc 180
tattctactc tccaatggtt ccgcattttt gggcagagct tcgcaatcat tatgcaacga 240
gtggtttgaa aagcgggtac aatattggga gtaccgatgg gtttctccct gtcattgggc 300
ctgttatatg ggagtcggag ggtcttttcc gcgcttatat ttcttcggtg actgatgggg 360
atggtaagag ccataaagta ggatttctaa gaattcctac atatagttgg caggacatgg 420
aagattttga tcc 433

<210> 128
<211> 803
<212> DNA
<213> Chlamydia trachomatis

<400> 128
atctattaat taatagcaag cttgaaacta aaaacctaat ttatttaaag ctcaaaataa 60
aaaagagttt taaaatggga aattctggtt tttatttgta taacactgaa aactgcgtct 120
ttgctgataa tatcaaagtt gggcaaatga cagagccgct caaggaccag caaataatcc 180
ttgggacaac atcaacacct gtcgcagcca aaatgacagc ttctgatgga atatctttaa 240
cagtctccaa taattcatca accaatgctt ctattacaat tggtttggat gcggaaaaag 300
cttaccagct tattctagaa aagttgggag atcaaatttct tgatggaatt gctgatacta 360
ttgttgatag tacagtccaa gatatttag acaaaatcaa aacagacoct tctctaggtt 420
tgttgaaagc ttttaacaac tttccaatca ctaatcaaat tcaatgcaac gggttattca 480
ctcccagtaa cattgaaact ttattaggag gaactgaaat aggaaaattc acagtcacac 540
ccaaaagctc tgggagcatg ttcttagtct cagcagatat tattgcatca agaatggaag 600
gcggcgttgt tctagctttg gtacgagaag gtgattctaa gccctgcgcg attagttatg 660
gatactcatc aggcattcct aatttatgta gtctaagaac cagtattact aatacaggat 720
tgactccgac aacgtattca ttacgtgtag gcggtttaga aagcggtgtg gtatgggtta 780
atgccctttc taatctcgtg ccg 803

<210> 129
<211> 842
<212> DNA
<213> Chlamydia trachomatis

<400> 129
tgggaatgtc gaagaatacg attacgttct cgtatctata ggacgccgtt tgaatacaga 60
aaatattggc ttggataaag ctggtgttat ttgtgatgaa cgcggagtca tccctaccga 120
tgccacaatg cgcacaaacg tacctaacat ttatgctatt ggagatatca caggaaaatg 180
gcaacttgcc catgtagctt ctcatcaagg aatcattgca gcacggaata tagctggcca 240
taaagaggaa atcgattact ctgccgtccc ttctgtgatc tttaccttcc ctgaagtcgc 300
ttcagtaggc ctctccccaa cagcagctca acaacaaaaa atcccccgtca aagtaacaaa 360
attcccattt cgagctattg gaaaagcggt cgcaatgggc gaggccgatg gatttgcagc 420
cattatcagc catgagacta ctcagcagat cctaggagct tatgtgattg gccctcatgc 480
ctcatcactg atttccgaaa ttaccctagc agttcgtaat gaactgactc ttccttgtat 540
ttacgaaact atccacgcac atccaacctt agcagaagtt tgggctgaaa gtgcgttgtt 600
agctgctgat acccattac atatgcccc tgctaaaaaa tgaccgattc agaatctcct 660
actcctaaaa aatctatacc cgccagattc cctaagtggc tacgccagaa actcccttta 720
gggcgggtat ttgctcaaac tgataatact atcaaaaata aagggcttcc tacagtctgt 780
gaggaagcct cttgtccgaa tcgcaccat tgttggtcta gacatacagc tacctatcta 840
gc 842

<210> 130
<211> 813
<212> DNA

<213> Chlamydia trachomatis

<400> 130
aaaatacttt gagctgcaca agctcccccc tgttctagag aagaacatga tgcaaattcc 60
aatccaccct taatctttcc aaagataaga tcttctgtag aatataaagc cgctccagac 120
aaagaagctt tcacgtcagt taatgtgatt ccagccttac tactatcccc aacaaaagca 180
atacctaaaa aagattctcc gtcacgagga gaatcaaggt tgctgctcgt aaaactacaa 240
attaaccctt gggaagagac ttgatcctgt tggtccacac ctttggaaaac tacgggattg 300
gttactgaga acaaagtact ttgctctacc ttaccgggaa gagtatccgc atctttctct 360
tggaaagaac ttggatctcc tacaattaac ctatactgtc cttcagcctg actatcttta 420
gacccaacga atagatctcg aatttggtct aacaataaaa ccgcttgagg gcctacatat 480
accagctcat ttacagactg tcctccagca tgaagatcta cgcaactagc taaccccta 540
acagaggcaa ggatagctgc tactacagac aaagaaaact tagaacaggt gcttttttata 600
tctttctcgg aactcatttc aaacctgcga aatagcactt ttttgacaaa ctagcgtacc 660
gaaacaatcg gtccaacaac gcgttctgcc tatgatttca caaagacaaa acgacccata 720
gacaagctcc agagacgaca ttagagcttt agaccgtgga atgtacaatg ctgactgctt 780
tttgagaaag atttttata aagaacaggc cct 813

<210> 131
<211> 1947
<212> DNA
<213> Chlamydia trachomatis

<400> 131
tctttgcct atagagcaat ctcttatcat tgggtctgat ccaccagact atttcttcta 60
gatagagatt ctactacccc atccatggca ttcaacctct catcagtaaa cactttatta 120
gagttgttta tctgcccatc atcgatgata tcttctgaag tctttaatac cttcttacat 180
aagatccatc tctcggaga acagtgtcct tctatggata aaattcctac gcagatattc 240
acgcatccca aaatagcagg aatacctaga tagatggcat ttacaaacga agctgccgaa 300
actaggaata tcaaagcagt aatcactaaa agtagtccta tcaccactaa tcccaccttа 360
aatgcagtgg aagatagaag attcgatata cgctctttca gtgttaatgg tgcagaacta 420
gtggaaatat cctgtgccga attggaagat ccagctcctt gaacaacggg tacagtgctc 480
atatttttaca ttcctttttt ggttgtgagc agggagtcta cacaaacact tatttttttc 540
aaaaaccgct ctagaatatg ctctgagacc gaaaatgaac tcttttattt tcatatagat 600
aacaaaaaaa agcgccccag gaatccctgg acggcaccta cacatcgata aaatcaaaga 660
ttaatagatg tgtgtattct ctgtatcaga aactggaaca gtcaatgtat cggaagaaag 720
aatcgcttcc ccacgagcat ctccagctga tactgctttc aatgttacag aaaactctac 780
agtttcttta gaacctaatc taggtaacga atcgaatact actgttattgc ctgtaatcgt 840
tcctttagtt ggtccagaga aggatacagg ttgcagttct ttagagaatt taagcattaa 900
agaaacattt gtatcttctg cagaacctct gttggtgaca caaatacggt aaacagtatt 960
ttctcctaca caaacagggt cacaagtatc tactacgcac atatgagtag cagcaactcc 1020
tttccagtaa gttgtcgctt ctgcgcaaga agtacaagta ccacagtcag agcagctctt 1080
cacaacaaca ttatttgtga attgtccagg agtttgtgct cttactagaa ctttatactg 1140
tagagactct ccaggattca gttctttcac agtccaaact actttattac aagaaatttg 1200
agctcctgca gcttcaagaa ctgtgactcc gggagaaaga gtgtcttcaa cgacgacatc 1260
tcgcaacaca agatctccag gattggaaac ggagatcaca tattctacag gcttacaaac 1320
ataagaccaa tctgctcctg caatacttac ttgtacgcaa ggctcattga tcacagttgt 1380
tacgcttgct gtatttttat gtcctccaca gtaagaaacc gttgctatat tggtagcacg 1440
accacgttta agcggacaaa actctacagt aattgttctg tgctctccag gttgcatatc 1500
tccaagagta aacgtcagta cacgctgtcc agaagagtga gcgtaaccat ctggaacagg 1560
attttcaaca acaacgttac gagctattgc tgttccttgg ttcactacat taattttgta 1620
aactactggg caacgcaaac aagcattctc tgggccttct tgtttaacac agatagcagg 1680
ttgtccacat tttgtaaccg aacggatctc tggacaagcg catactgttg cagctgtaaa 1740
gcagcaacct tctttaagag gttttaccca tacagtaatt ttactctttt cgccttgtcc 1800
taagcggtca attttccaaa ctagcttacc atcagcagta ggagttgtcg ctggatcact 1860
gcgtacgaac tctgcttcac atggttaatg ctgagtaatg ataacatcaa cacaatccct 1920
tttacctgta gcagtaattt caatagg 1947

<210> 132
<211> 1278

<212> DNA
<213> Chlamydia trachomatis

<400> 132
gataacaaaa aaaagccgcc caggaatccc tggacggcac ctacacatcg ataaaatcaa 60
agattaatag atgtgtgtat tctctgtatc agaaactgga acagtcaatg tatcggaaga 120
aagaatcgct tcccacgag catctccagc tgatactgct ttcaatgtta cagaaaactc 180
tacagtttct ttagaacta atctaggtaa cgaatcgaat actactgtat tgcctgtaat 240
cgttcctta gttggtccag agaaggatac aggttgcagt tctttagaga atttaagcat 300
taaagaaaca tttgtatctt ctgcagaacc tctgttggtg acacaaatac ggtaaacagt 360
attttctcct acacaaacag ggtcacaagt atctactacg cacatatgag tagcagcaac 420
tcctttccag taagttgtcg cttctgcgca agaagtacaa gtaccacagt cagagcagct 480
cttcacaaca acattatttg tgaattgtcc aggagtttgt gctcttacta gaacttttat 540
ctgtagagac tctccaggat tcagttcttt cacagtccaa actactttat tacaagaaat 600
ttgagctcct gcagcttcaa gaactgtgac tccgggagaa agagtgtctt caacgacgac 660
atctcgcaac acaagatctc caggattgga aacggagatc acatatttcta caggcttaca 720
aacataagac caatctgctc ctgcaatact tacttgtacg caaggctcat tgatcacagt 780
tgttacgctt gctgtatttt tatgtcctcc acagtaagaa acogttgcta tattggtagc 840
acgaccacgt ttaagcggac aaaactctac agtaattgtt ctgtgctctc caggttgcat 900
atctccaaga gtaaacgtca gtacacgctg tccagaagag tgagcgtaac catctggaac 960
aggatttta acaacaacgt tacgagctat tgctgttcct tggttcacta cattaatttt 1020
gtaaactact gggcaacgca aacaagcatt ctctgggcct tcttgtttaa cacagatagc 1080
aggttgtcca cattttgtaa ccgaacggat ctctggacaa gcgcatactg ttgcagctgt 1140
aaagcagcaa ccttctttaa gaggttttac ccatacagta attttactct tttcgccttg 1200
tcctaagcgg tcaattttcc aaactagctt accatcagca gtaggagttg tcgctggatc 1260
actgcgtacg aactctgc 1278

<210> 133
<211> 916
<212> DNA
<213> Chlamydia trachomatis

<400> 133
atggcgacaa tttaacgatt accggacaaa accatacatt atcatttaca gattctcaag 60
ggccagttct tcaaaattat gccttcattt cagcaggaga gacacttact ctgaaagatt 120
tttcgagttt gatgttcttcg aaaaatgttt cttgcggaga aaagggaatg atctcaggga 180
aaacccgtgag tatttccgga gcaggcgaag tgatttttg ggataactct gtgggtatt 240
ctccttttgtc tattgtgcca gcatcgactc caactcctcc agcaccagca ccagctcctg 300
ctgcttcaag ctctttatct ccaacagtta gtgatgctcg gaaagggtct attttttctg 360
tagagactag tttggagatc tcaggcgtca aaaaggggt catgttcgat aataatgccg 420
ggaattttgg aacagttttt cgaggtaata gtaataataa tgctggtagt gggggtagtg 480
ggtctgctac aacaccaagt tttacagtta aaaactgtaa agggaaagtt tctttcacag 540
ataacgtagc ctcctgtgga ggcggagtag tctacaaagg aactgtgctt ttcaaagaca 600
atgaaggagg catattcttc cgagggaaca cagcatacga tgatttagtg attcttgctg 660
ctactagtcg ggatcagaat acggagacag gaggcggtgg aggagttatt tgctctccag 720
atgattctgt aaagtttgaa ggcaataaag gttctattgt ttttgattac aactttgcaa 780
aaggcgaggg cggaagcatc ctaacgaaag aattctcttct tgtagcagat gattcggttg 840
tctttagtaa caatacagca gaaaaaggcg gtggagctat ttatgctcct acgtatcgat 900
ataagcacga atggag 916

<210> 134
<211> 751
<212> DNA
<213> Chlamydia trachomatis

<220>
<221> misc_feature
<222> (1)..(751)
<223> n = A,T,C or G

<400> 134
agcctctggc gaaggagagc cataaaaagt gcctaccagc ggagaaacaa taaaatctcc 60
ctgagcaggc acctcacttt ctttcttctc gatactctct ttaacaatag gattcccaag 120
gttttgatct gaggataagt tttgaaatcc agcaaacagt ctgttatcat aaaagactgg 180
ctcctgaata ctggggactg tatccttttc taactctaac tccaaacctt cacgcttgat 240
aacaatgcgc ttcacgtgcc gaattcggca cgaggctctt tcttacgagg atctcgagtc 300
aagaagcctt gagccttcaa ttcttgcttc atgtcttctt tctcttgcag aacagctcta 360
gctaaaccca atcgagtagc aataacctga ccttgaaccc ctcctccact tactcggata 420
atcaaatcga aactgttgac atcaccgagc attctgagcg gagctaagat ggttgctctt 480
tgaacttcaa gagggaaata ttgctctaaa gtctttccat ttacgtcaat tttccattc 540
ccagaacgaa gacgaacgca cacctgcttt cttctgcctg ttgcaacaga ctcttgtatc 600
atattctttg tcacaaatta ccccaaatta cgcgtctaaa acaattggtt tgatagcttc 660
atactgtgcg taagaactac ctttcaaaac tcttaaagat ttcatttgac gtcttccaag 720
ttttgtttta ggcaacattc nttaacagca t 751

<210> 135
<211> 410
<212> DNA
<213> Chlamydia trachomatis

<400> 135
ataatccaga ctcttcctca tctggagata gcgctggaga ctctgaagaa ctgactgaga 60
cagaagctgg ttctacaaca gaaactccta ctttaatagg aggaggtgct atctatggag 120
aaactgttaa gattgagaac ttctctggcc aaggaatatt ttctggaaac aaagctatcg 180
atacacacac agaaggctcc tcttccaaat ctgacgtcct cggaggtgcg gtctatgcta 240
aaacattgtt taatctcgat agcgggagct ctagacgaac tgtcaccttc tcggggaata 300
ctgtctcttc tcaatctaca acaggtcagg ttgctggagg agctatctac tctcctactg 360
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<210> 136
<211> 2719
<212> DNA
<213> Chlamydia trachomatis

<400> 136
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ccggtgcatt cataaacagc ttcctcgtaa tggtgtagat tgttcggggt atattcaact 180
actttaccaa gtcacaggaa gaaatatccc tcgcaatgct agagatcaat acagagactg 240
ttctccagta aaagatttct cgtctctacc tataggagga cttatcttcc tcaagaaagc 300
aagcacggga caaatcaacc atgttatgat gaaaatctcg gagcatgaat tcattcatgc 360
tgcggaaaaa atagggaaag tagaaaaagt aatcctagga aataggggctt tctttaaagg 420
gaatctatcc tgctcattag gtgaaccgcc tatagaagct gttttggcg ttcctaaaaa 480
tagaaaagcc ttctttttgaa agaaggcttt tctgaaacgc actccaatat atggacaagc 540
aatagcttat cgtttggaga attggaaact cttacgagct ttcttacgac cgtatttttt 600
acgctctttc ttacgaggat ctcgagtcaa gaagccttga gccttcaatt cttgcttcat 660
gtcttctttc tcttgcagaa cagctctagc taaacccaat cgagtagcaa taacctgacc 720
ttgaacccct cctccactta ctcggataat caaatcgaaa ctgttgacat caccgagcat 780
tctgagcgga gctaagatgg ttgctctttg aacttcaaga gggaaatatt gctctaaagt 840
ctttccattt acgtcaattt ttccattccc agaacgaaga cgaacgctag aaacagcctg 900
ctttcttctg cctgttgcaa cagactcttg tatcatattc tttgtcacaa attaccccaa 960
attacgcgtc taaaacaatt ggtttgatag cttcatactg tgcgtaagaa ctacctttca 1020
aaactcttaa agatttcatt tgacgtcttc caagttttgt tttaggcaac attcctttaa 1080
cagcatgctc gataacataa gcaggctttc gcgcaatcat gttttcaaaa ggaacttctc 1140
gcatcccaga aataaagcct gtgtaatagt gatacacttt ctgagttcct tttgcgccag 1200
tcaaacgcac tttctcagca ttgatcacaa tgacacatc tcccatcgct acgtgaggag 1260
taaaagtcac cttatgctta cctctcagga tcttcgcaac ttctgaagat aatctcccta 1320
aggtcttccc ttcagcatta actacatacc aggctttgtt tcgatcgtcc gaagccttag 1380

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gaatttagaa aagaaaaaat ttcaaaaaga tctcttttct ttttgccttc aaaaacagcc 1560
ttacacttct atacttcttt cgaaaaaata ttttagggaa gttcttgaat catgatttac 1620
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tggaatcata ataaggaggt atcgcataac cactgtagca agccccgtct actcgcacag 1920
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tccgagctaa atccactgct actttccaa ctttagctcg catttctgga gttaaaatag 2280
gacttggagt ctcttctatt aatttttgcc gacgcctttg tactgacaat ctcgttctcc 2340
aagatacacg taatttccgt gcttatctcc aattacttga acttctaaat gtcttggatt 2400
ttcaataaat ttttcaatat acacgtcagg attattaaat cccgcttctg ctccagccag 2460
agcggcagta aaagccctat agaattcgtc tttttctcta acaatccgta ttcctcgtcc 2520
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ccctccacc tcatccttca ctacaccttc agatccaggg attacaggcc acttaatctt 2640
tttagccaac tgcttagctg cgacttttatc tcccatagtc gctatcgact cagcactagg 2700
accgataaat ctcgtgccg                                          2719
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<210> 137
<211> 2354
<212> DNA
<213> Chlamydia trachomatis

<400> 137

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ctaaatgtct ctaagtaagg atgtttttag gggaaatagc gattttcagt gttgagaagc 180
ttagttacaa gacaataaac aaggctaaga aaaacctttc ttagccttgt ttctcaacga 240
atcgcctata gaagactaat cttccagcgt tgccctatgg ctcagcttca actggccttt 300
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gacttctacg aatactccaa atgttgcgat agatgtaaca cggccattat aaacttaacc 480
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ggtgcttgca gctatgttaa tgacgccgtc atcattgatg tcaacttgcg caccagaacg 600
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cagaacctgt gtcataagat taaggatatg actacgccct tgtttagctt gcgctagagc 780
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acctttagct gttccggcta ctttaaagtc catatctcct agatgatctt ctatacggga 900
aatatcagac aagatgatgg cttgatctcg atctaagatt aagcccatag caatacctgc 960
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aaccaagtta gcaatcaacgg attcctctaa ttctttcgag gcttgctcta gagcttcttt 1560
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tagaacttgc tcttctgtta aaaagtcgca atgtccttca atcattaaaa ctgcagaagc 1800
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catggagttg tcct                                                   2354

<210> 138
<211> 898
<212> DNA
<213> Chlamydia trachomatis

<400> 138
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ccggaacagc taaaggtatt acagctttcc aaatggatat caagatagag ggaatcactc 120
ataagattat ggagcaagct ctagcgcaag ctaaacaagg gcgtagtcat atccttaatc 180
ttatgacaca ggttctggcc tcccctaagg gaactgtttc taaatatgct ccgcgcattg 240
aaactatgca gatcaatacc tcaaaaatcg caacggtcat tggtcccgga ggaaaacaaa 300
tccgtcaaat tatcgagcgt tctggtgcgc aagttgacat caatgatgac ggcgtcatta 360
acatagctgc aagcacccaa gaatcgatta acaaagctaa agaacttatc gaaggattaa 420
ctggagaagt tgaagtcggt aaagtttata atggccgtgt tacatctatc gcaacatttg 480
gagtattcgt agaagtcctc ccaggaaaag aagggctctg tcatatttct gaattgtcta 540
atcaaaaagt agacaatatc tctggcttcg tcaaagaagg agacaagctt gctgttaaac 600
tccttagcat taacgaaaaa ggccagttga agctgagcca tagggcaacg ctggaagatt 660
agtcttctat aggcgattcg ttgagaaaca aggctaagaa aggtttttct tagccttgtt 720
tattgtcttg taactaagct tctcaacact gaaaatogct atttccccta aaaacatcct 780
tacttagaga catttagtta gacgctagct ttcctcacac acaaaaaaag agagccctaa 840
taaaagggct ctctcctaaa cctccagaag ggaagttatg tgctagtatt aaacttca   898


<210> 139
<211> 660
<212> PRT
<213> Chlamydia trachomatis

<400> 139
Met His His His His His His Met Glu Ser Gly Pro Glu Ser Val Ser
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Ser Asn Gln Ser Ser Met Asn Pro Ile Ile Asn Gly Gln Ile Ala Ser
            20                  25                  30
Asn Ser Glu Thr Lys Glu Ser Thr Lys Ala Ser Glu Ala Ser Pro Ser
        35                  40                  45
Ala Ser Ser Ser Val Ser Ser Trp Ser Phe Leu Ser Ser Ala Lys Asn
    50                  55                  60
Ala Leu Ile Ser Leu Arg Asp Ala Ile Leu Asn Lys Asn Ser Ser Pro
65                  70                  75                  80
Thr Asp Ser Leu Ser Gln Leu Glu Ala Ser Thr Ser Thr Ser Thr Val
                85                  90                  95
Thr Arg Val Ala Ala Lys Asp Tyr Asp Glu Ala Lys Ser Asn Phe Asp
            100                 105                 110
Thr Ala Lys Ser Gly Leu Glu Asn Ala Lys Thr Leu Ala Glu Tyr Glu
        115                 120                 125
Thr Lys Met Ala Asp Leu Met Ala Ala Leu Gln Asp Met Glu Arg Leu
    130                 135                 140
Ala Asn Ser Asp Pro Ser Asn Asn His Thr Glu Glu Val Asn Asn Ile
145                 150                 155                 160
Lys Lys Ala Leu Glu Ala Gln Lys Asp Thr Ile Asp Lys Leu Asn Lys
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                165                 170                 175
Leu Val Thr Leu Gln Asn Gln Asn Lys Ser Leu Thr Glu Val Leu Lys
            180                 185                 190
Thr Thr Asp Ser Ala Asp Gln Ile Pro Ala Ile Asn Ser Gln Leu Glu
        195                 200                 205
Ile Asn Lys Asn Ser Ala Asp Gln Ile Ile Lys Asp Leu Glu Arg Gln
    210                 215                 220
Asn Ile Ser Tyr Glu Ala Val Leu Thr Asn Ala Gly Glu Val Ile Lys
225                 230                 235                 240
Ala Ser Ser Glu Ala Gly Ile Lys Leu Gly Gln Ala Leu Gln Ser Ile
                245                 250                 255
Val Asp Ala Gly Asp Gln Ser Gln Ala Ala Val Leu Gln Ala Gln Gln
            260                 265                 270
Asn Asn Ser Pro Asp Asn Ile Ala Ala Thr Lys Glu Leu Ile Asp Ala
        275                 280                 285
Ala Glu Thr Lys Val Asn Glu Leu Lys Gln Glu His Thr Gly Leu Thr
    290                 295                 300
Asp Ser Pro Leu Val Lys Lys Ala Glu Glu Gln Ile Ser Gln Ala Gln
305                 310                 315                 320
Lys Asp Ile Gln Glu Ile Lys Pro Ser Gly Ser Asp Ile Pro Ile Val
                325                 330                 335
Gly Pro Ser Gly Ser Ala Ala Ser Ala Gly Ser Ala Ala Gly Ala Leu
            340                 345                 350
Lys Ser Ser Asn Asn Ser Gly Arg Ile Ser Leu Leu Leu Asp Asp Val
        355                 360                 365
Asp Asn Glu Met Ala Ala Ile Ala Leu Gln Gly Phe Arg Ser Met Ile
    370                 375                 380
Glu Gln Phe Asn Val Asn Asn Pro Ala Thr Ala Lys Glu Leu Gln Ala
385                 390                 395                 400
Met Glu Ala Gln Leu Thr Ala Met Ser Asp Gln Leu Val Gly Ala Asp
                405                 410                 415
Gly Glu Leu Pro Ala Glu Ile Gln Ala Ile Lys Asp Ala Leu Ala Gln
            420                 425                 430
Ala Leu Lys Gln Pro Ser Ala Asp Gly Leu Ala Thr Ala Met Gly Gln
        435                 440                 445
Val Ala Phe Ala Ala Ala Lys Val Gly Gly Gly Ser Ala Gly Thr Ala
    450                 455                 460
Gly Thr Val Gln Met Asn Val Lys Gln Leu Tyr Lys Thr Ala Phe Ser
465                 470                 475                 480
Ser Thr Ser Ser Ser Ser Tyr Ala Ala Ala Leu Ser Asp Gly Tyr Ser
                485                 490                 495
Ala Tyr Lys Thr Leu Asn Ser Leu Tyr Ser Glu Ser Arg Ser Gly Val
            500                 505                 510
Gln Ser Ala Ile Ser Gln Thr Ala Asn Pro Ala Leu Ser Arg Ser Val
        515                 520                 525
Ser Arg Ser Gly Ile Glu Ser Gln Gly Arg Ser Ala Asp Ala Ser Gln
    530                 535                 540
Arg Ala Ala Glu Thr Ile Val Arg Asp Ser Gln Thr Leu Gly Asp Val
545                 550                 555                 560
Tyr Ser Arg Leu Gln Val Leu Asp Ser Leu Met Ser Thr Ile Val Ser
                565                 570                 575
Asn Pro Gln Ala Asn Gln Glu Glu Ile Met Gln Lys Leu Thr Ala Ser
            580                 585                 590
Ile Ser Lys Ala Pro Gln Phe Gly Tyr Pro Ala Val Gln Asn Ser Ala
        595                 600                 605
Asp Ser Leu Gln Lys Phe Ala Ala Gln Leu Glu Arg Glu Phe Val Asp
    610                 615                 620
Gly Glu Arg Ser Leu Ala Glu Ser Gln Glu Asn Ala Phe Arg Lys Gln
625                 630                 635                 640
Pro Ala Phe Ile Gln Gln Val Leu Val Asn Ile Ala Ser Leu Phe Ser
                645                 650                 655
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Gly Tyr Leu Ser
660

<210> 140
<211> 598
<212> PRT
<213> Chlamydia trachomatis

<400> 140
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Gly Asn Ser Arg Ile Pro Ser His Asn Gly Asp Gly Ser Asn Arg Arg
20 25 30
Ser Gln Asn Thr Lys Gly Asn Asn Lys Val Glu Asp Arg Val Cys Ser
35 40 45
Leu Tyr Ser Ser Arg Ser Asn Glu Asn Arg Glu Ser Pro Tyr Ala Val
50 55 60
Val Asp Val Ser Ser Met Ile Glu Ser Thr Pro Thr Ser Gly Glu Thr
65 70 75 80
Thr Arg Ala Ser Arg Gly Val Leu Ser Arg Phe Gln Arg Gly Leu Val
85 90 95
Arg Ile Ala Asp Lys Val Arg Arg Ala Val Gln Cys Ala Trp Ser Ser
100 105 110
Val Ser Thr Ser Arg Ser Ser Ala Thr Arg Ala Ala Glu Ser Gly Ser
115 120 125
Ser Ser Arg Thr Ala Arg Gly Ala Ser Ser Gly Tyr Arg Glu Tyr Ser
130 135 140
Pro Ser Ala Ala Arg Gly Leu Arg Leu Met Phe Thr Asp Phe Trp Arg
145 150 155 160
Thr Arg Val Leu Arg Gln Thr Ser Pro Met Ala Gly Val Phe Gly Asn
165 170 175
Leu Asp Val Asn Glu Ala Arg Leu Met Ala Ala Tyr Thr Ser Glu Cys
180 185 190
Ala Asp His Leu Glu Ala Lys Glu Leu Ala Gly Pro Asp Gly Val Ala
195 200 205
Ala Ala Arg Glu Ile Ala Lys Arg Trp Glu Lys Arg Val Arg Asp Leu
210 215 220
Gln Asp Lys Gly Ala Ala Arg Lys Leu Leu Asn Asp Pro Leu Gly Arg
225 230 235 240
Arg Thr Pro Asn Tyr Gln Ser Lys Asn Pro Gly Glu Tyr Thr Val Gly
245 250 255
Asn Ser Met Phe Tyr Asp Gly Pro Gln Val Ala Asn Leu Gln Asn Val
260 265 270
Asp Thr Gly Phe Trp Leu Asp Met Ser Asn Leu Ser Asp Val Val Leu
275 280 285
Ser Arg Glu Ile Gln Thr Gly Leu Arg Ala Arg Ala Thr Leu Glu Glu
290 295 300
Ser Met Pro Met Leu Glu Asn Leu Glu Glu Arg Phe Arg Arg Leu Gln
305 310 315 320
Glu Thr Cys Asp Ala Ala Arg Thr Glu Ile Glu Glu Ser Gly Trp Thr
325 330 335
Arg Glu Ser Ala Ser Arg Met Glu Gly Asp Glu Ala Gln Gly Pro Ser
340 345 350
Arg Val Gln Gln Ala Phe Gln Ser Phe Val Asn Glu Cys Asn Ser Ile
355 360 365
Glu Phe Ser Phe Gly Ser Phe Gly Glu His Val Arg Val Leu Cys Ala
370 375 380
Arg Val Ser Arg Gly Leu Ala Ala Ala Gly Glu Ala Ile Arg Arg Cys
385 390 395 400
Phe Ser Cys Cys Lys Gly Ser Thr His Arg Tyr Ala Pro Arg Asp Asp

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                405                 410                 415
Leu Ser Pro Glu Gly Ala Ser Leu Ala Glu Thr Leu Ala Arg Phe Ala
            420                 425                 430
Asp Asp Met Gly Ile Glu Arg Gly Ala Asp Gly Thr Tyr Asp Ile Pro
        435                 440                 445
Leu Val Asp Asp Trp Arg Arg Gly Val Pro Ser Ile Glu Gly Glu Gly
    450                 455                 460
Ser Asp Ser Ile Tyr Glu Ile Met Met Pro Ile Tyr Glu Val Met Asn
465                 470                 475                 480
Met Asp Leu Glu Thr Arg Arg Ser Phe Ala Val Gln Gln Gly His Tyr
                485                 490                 495
Gln Asp Pro Arg Ala Ser Asp Tyr Asp Leu Pro Arg Ala Ser Asp Tyr
            500                 505                 510
Asp Leu Pro Arg Ser Pro Tyr Pro Thr Pro Pro Leu Pro Pro Arg Tyr
        515                 520                 525
Gln Leu Gln Asn Met Asp Val Glu Ala Gly Phe Arg Glu Ala Val Tyr
    530                 535                 540
Ala Ser Phe Val Ala Gly Met Tyr Asn Tyr Val Val Thr Gln Pro Gln
545                 550                 555                 560
Glu Arg Ile Pro Asn Ser Gln Gln Val Glu Gly Ile Leu Arg Asp Met
                565                 570                 575
Leu Thr Asn Gly Ser Gln Thr Phe Arg Asp Leu Met Lys Arg Trp Asn
            580                 585                 590
Arg Glu Val Asp Arg Glu
        595
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